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# IMMUNOLOGICAL STUDIES ON THE THREE PEACH DISEASES, YELLOWS, ROSETTE, AND LITTLE PEACH[1]

L. O. KUNKEL

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## INTRODUCTION

It has been shown that plants of *Nicotiana sylvestris* Spegaz. and Comes affected by ordinary tobacco mosaic or by mild strains of this disease become immune from aucuba mosaic of tomato, which is caused by a severe strain of the tobacco-mosaic virus (4). Similar plants affected by other virus diseases, such as tobacco ring spot or cucumber mosaic, are not protected against aucuba mosaic. It likewise has been shown that zinnia plants affected by ordinary cucumber mosaic or by mild strains of this disease are immune from infection by a severe strain of cucumber-mosaic virus, but that similar plants having other virus diseases such as tobacco ring spot or tobacco mosaic are not protected against the severe strain of cucumber mosaic (5). These immunity reactions are specific, and it is, therefore, possible by inoculations to appropriate test plants to determine quickly and accurately whether any new disease belongs to either the tobacco-mosaic or the cucumber-mosaic group. If immunity reactions are equally specific for other virus diseases of plants, they should be useful in detecting relationships between these maladies.

Three virus diseases of peach, yellows, rosette, and little peach, are prevalent in the eastern United States. Yellows, which was first observed near Philadelphia in 1791, spread gradually northward through the New England states and into Canada. It spread southward into Delaware, Maryland, Virginia, West Virginia, and North Carolina. Little peach and rosette were discovered about 100 years after yellows began to attract attention. Little peach has much the same geographical range as yellows. Rosette is somewhat more southern in its distribution than either of the other diseases. However, it occurs apparently as far north as Michigan (1). The three diseases are alike in certain respects. They all cause stunting,

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the abnormal production of secondary shoots, and yellowing of mature leaves. None of them can be transmitted mechanically except by tissue transplantation. Yellows is more severe than little peach, and rosette more severe than yellows. It was thought that the three diseases might be caused by different strains of the same virus, and that evidence of such a relationship might be obtained from cross-immunity tests similar to those already described for the different strains of tobacco- and cucumber-mosaic diseases. Such tests have been made, and it is the purpose of this paper to report the results.

## MATERIALS AND METHODS

Potted seedling peach trees grown in greenhouses from seed obtained from a commercial dealer at Germantown, Pennsylvania, were used in all experiments. Most of the trees were uniform in appearance. Any that showed unusual individual variations were discarded. No trees were found that proved resistant to any one of the three diseases. Except for slight color variations, the symptoms of each disease were essentially identical in different seedlings. All trees were held in unheated cold frames during a portion of each winter in order that they might undergo the necessary period of dormancy. Noninoculated control trees were provided in all experiments. They were not necessary as a check against accidental infections, for such infections do not occur in greenhouse-grown seedlings. The controls were found to be useful in making comparisons between diseased and healthy trees. The yellows virus was obtained from Dr. T. F. Manns, of the Delaware Agricultural Experiment Station, the rosette virus from County Agent Mr. W. C. Johnstone, Paducah, Kentucky, and the little-peach virus from a commercial orchard near Clinton, New Jersey. Inoculations were by buds transplanted to the stems of the trees and, since location of point of inoculation proved to be important, a record was kept of the approximate height at which the buds were inserted. Practically all transplanted buds lived, and all that lived transmitted disease. Buds from yellows trees, from little-peach trees, and from rosette trees are designated as "yellows buds," "little-peach buds," and "rosette buds," respectively.

## SYMPTOMS

The symptoms of each of the three diseases are so well known and so adequately treated in the literature that it will not be necessary to describe them at length here. Since, however, there is evidence that distinct strains of at least two of the diseases, little peach and yellows, are prevalent in nature, and since certain symptoms either have not been mentioned previously or have not been emphasized sufficiently, a brief description of the diseases used in these experiments will be given. All of the symptoms to be described appeared in young seedlings growing under greenhouse conditions.

## Little Peach

The first visible symptom in trees to which little peach has been transmitted is distortion of young leaves at the tips of affected branches. The surfaces of these leaves are rugose at this stage in their development. As the affected leaves approach maturity, their surfaces may become as smooth as those of leaves on healthy trees. Another early symptom, but one that does not appear in all trees, is the production of an abnormally large number of short branches along the main stem, especially near the soil level. These branches show a more upright habit of growth than do branches from

FIG. 1. Two branches from peach trees of the same age. The branch on the right shows the twiggy type of growth characteristic of little-peach disease; the branch on the left is healthy.

healthy stems, but they do not take a position so near the vertical as is characteristic of similar branches on yellows trees. The disease causes shortening of internodes and general stunting of trees. Leaves and young branches on newly-infected trees are usually a somewhat deeper green color than is normal for healthy trees. After the disease has become chronic, mature leaves turn slightly yellow and a twiggy growth, such as is shown in figure 1, develops along the branches. Trees that have been diseased for 2 or 3 years produce an abnormally small number of new shoots following a period of dormancy.

FIG. 2. Two peach leaves from the same tree. The leaf on the left shows clearing-of-veins caused by the yellows virus, while the leaf on the right is normal.

## Yellows

An early symptom of the yellows disease is clearing-of-veins in leaves near the tips of young branches. The clearing is not so marked as to be conspicuous, but can usually be seen easily if observations are made at the proper time on rapidly-growing trees. Figure 2 shows 2 leaves of approximately the same size and age taken from the same tree 68 days after it was inoculated with yellows virus. The clearing-of-veins symptom shown by the leaf on the left first became visible about 3 weeks before the picture was taken. The leaf on the right is healthy in appearance.

FIG. 3. Two peach seedlings of the same age. The tree that has produced the willowy type of growth characteristic of yellows in its late stages was inoculated 9 months before the photograph was taken. The tree on the right is healthy.

A second early symptom appears in very young leaves as they develop at the tips of infected branches. Such leaves, when from 6 to 10 mm. long, stand straight in branches that are healthy, but bend over to form sickle-shape structures in branches that are diseased. The production of numerous thin upright-growing shoots bearing small slightly chlorotic leaves is a conspicuous symptom of the yellows disease after it is well established. A healthy and a yellows tree grown from seeds planted on the same date are shown in figure 3. Although the disease is invariably lethal, young trees frequently live for 1 to 2 years after their infection.

### Rosette

Trees infected by the rosette virus may show either of 2 distinct types of symptoms. They may wilt suddenly and die, as in the case of the tree shown in figure 4, or they may develop the well-known rosette type of growth pictured in figure 5. This growth is characterized by slight elongation of stems and branches and by progressive dwarfing of the leaves produced after infection. Clearing-of-veins and thickening of veins accompany the development of other rosette symptoms. Young leaves and twigs of rosette trees are dark green. The severity of the disease varies with the season of the year, the point of inoculation, and the age of the tree. If rapidly-growing, young trees become infected from inoculations made some distance above the soil level during the spring and early summer, they almost always wilt and die without producing rosettes. If, however, the trees are inoculated at the ground level, they usually produce the rosette type of growth, even though they are young and growing rapidly. If trees are large and growing slowly, they do not wilt without first producing rosettes, even when inoculations are made in their tops. Trees infected shortly before they become dormant invariably produce rosettes when growth is resumed after the rest period. All rosette trees wilt and die after a short period of time. They seldom live for more than a few months.

## EXPERIMENTAL

The cross-immunity experiments reported below were designed to show whether trees infected with any one of the 3 peach viruses would acquire immunity from either of the other 2.

### Inoculation of Yellows Trees and Little-Peach Trees with Rosette Virus

Experiments in which trees having yellows and trees having little peach were inoculated with rosette virus have brought no evidence that either the yellows or the little-peach disease gives any protection against rosette. When rosette buds are inserted in yellows trees or in little-peach trees, the

trees come down with rosette disease as promptly as do healthy ones. When subinoculations are made from any part of these trees to healthy seedlings, the rosette disease is transmitted. This proves that rosette virus quickly invades tissues infected by either yellows or little peach. The rosette disease affects trees so severely that it has not been possible to determine whether either the yellows disease or the little-peach disease is transmitted by these subinoculations, but it is clear that neither yellows nor little peach gives protection against rosette.

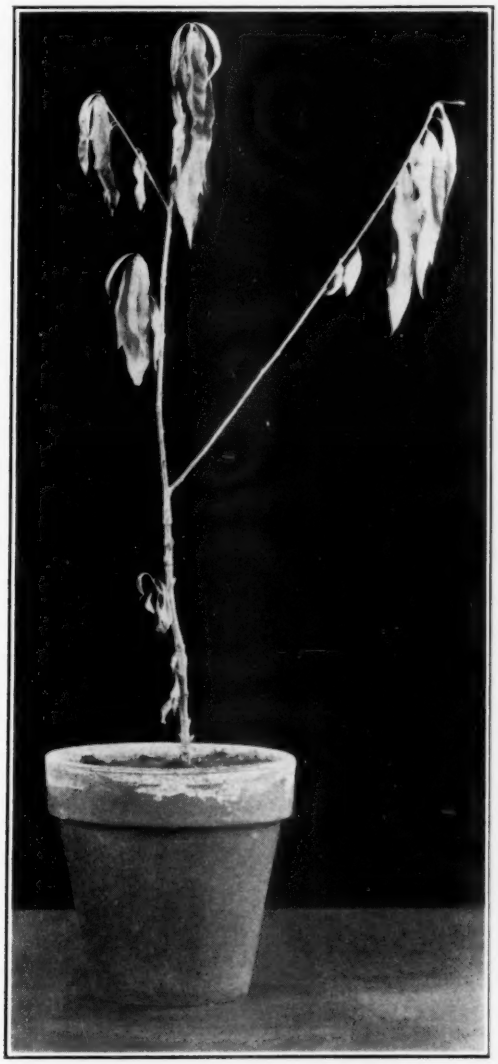

FIG. 4. A peach seedling that shows wilting without other rosette symptoms.

In one of these experiments, 5 trees belonging to a group of 10, that had had yellows for about 6 months and were thoroughly diseased, and 5 trees of another group of 10, that had had little peach for the same period of time and were diseased throughout, were inoculated with rosette virus by the insertion of rosette buds in their tops. At the same time, 5 out of a group of 10 healthy trees that were of the same age as the yellows trees and little-peach trees were similarly inoculated. The trees were held under observation during a period of 4 months following the rosette inoculations. All of the trees began to show the symptoms of rosette disease in about 5 weeks

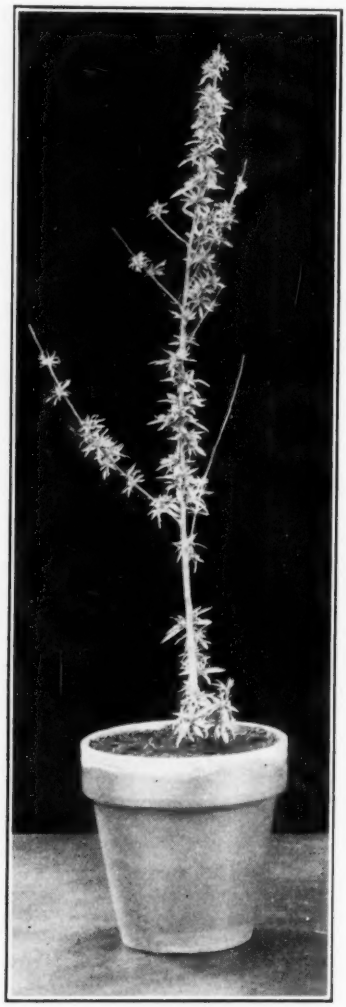

FIG. 5. A tree showing the usual rosette symptoms.

after they were budded. The rosette type of growth was produced at the tips of branches and the bark of all trees became a deeper green color than it had been previous to the rosette inoculations. Subinoculations to healthy trees from each of the 5 yellows trees and from each of the 5 little-peach trees 4 months after they were inoculated with rosette virus transmitted rosette disease. Symptoms developed by the trees gave no indication of transmission of yellows or little peach. This is not interpreted as evidence that the yellows and little-peach diseases were not transmitted together with the rosette disease. The symptoms of both yellows and little peach appear in the new growth that is produced after trees become infected. Rosette checks growth to such an extent that the symptoms of these diseases cannot develop. The experiments prove that rosette virus readily invades trees that have either the yellows or the little-peach disease, and that neither disease gives protection against rosette.

Reciprocal tests in which trees having rosette disease were inoculated with yellows virus or with little-peach virus have not yielded evidence that these viruses invade rosette tissues. This is not interpreted as proof that rosette gives protection against yellows and little peach, for the rosette trees never grow sufficiently to develop symptoms of either yellows or little peach. Yellows buds and little-peach buds transplanted to rosette trees take on the same deep green that is characteristic of the bark of rosette trees. All shoots produced by such buds show rosette symptoms. These tests bring further evidence that rosette virus readily invades tissues affected by either the yellows or the little-peach disease.

### Inoculation of Trees Having Little Peach with Yellows Virus

Many experiments have been made in which trees having the little-peach disease were inoculated by inserting in their stems or branches buds from yellows trees. The yellows buds have lived and have, in many instances, produced shoots, but in no case have they transmitted the yellows disease. In a typical experiment, 10 healthy young trees about 3 feet tall and of the same age were inoculated with little-peach virus by the insertion of a bud in each tree at a point about 15 inches above the soil level. Six similar trees were left uninoculated. The buds all lived and transmitted the little-peach disease to the trees. Two months after inoculation of these 10 trees, 8 of them were reinoculated by the insertion of a yellows bud in the stem of each at a point about 7½ inches above the soil level. Two of the healthy trees were similarly inoculated with yellows at the same time. The other 4 healthy trees were not inoculated. All of the buds lived. Yellows was transmitted to the 2 healthy trees, but was not transmitted to any of the 8 trees having the little-peach disease. In order to subject the immunity of the trees to a severe test, they were inoculated with yellows a second time

by the insertion of a yellows bud in the stem of each tree at a point about 12 inches above the soil level 2 months after the first yellows inoculations were made. A yellows bud was inserted in the stem of each of 2 of the healthy trees at this time. The other 2 healthy trees were left uninoculated. All of the buds lived. The 10 trees inoculated with little-peach virus showed the symptoms of little-peach disease in less than 2 months after they were budded, and they continued to show these symptoms until the experiment was ended, 15 months later. Although 8 of these trees were inoculated with yellows on 2 occasions, they exhibited only symptoms of little-peach disease. Their appearance was in every respect like that of the 2 trees that were inoculated with little-peach virus alone. The 4 trees inoculated only with yellows virus developed typical symptoms of yellows within 50 days after they were budded. The experiment proves that trees having little-peach disease are immune from yellows.

### Shoots from Yellows Buds Used in the Inoculation of Trees Having Little-peach Disease

Mention already has been made of the fact that yellows buds transplanted to trees having the little-peach disease sometimes produce shoots. It is interesting to note that such shoots never show any of the symptoms of yellows, but invariably show typical symptoms of little-peach disease. When subinoculations are made from these shoots to healthy trees, the symptoms obtained are those of little peach and not those of the yellows disease. These statements are supported by results from a number of different experiments, one of which will be described.

A little-peach bud was inserted in the stem of each of 5 healthy young peach trees about 4 feet tall at points approximately 2 feet above the soil level. Two similar healthy trees were left uninoculated. The buds all lived and the trees into which they were transplanted all showed typical symptoms of little-peach disease in less than 2 months after their inoculation. Eight months after the 5 trees were inoculated with little-peach virus, they were reinoculated by the insertion of 5 yellows buds in the stem of each tree at points less than 2 feet above the soil level. At the same time 5 yellows buds were inserted in the stem of one of the healthy trees. The other healthy tree was left uninoculated. Two weeks after the insertion of yellows buds, the trees were cut back in order to force the transplanted buds into growth. All shoots except those from yellows buds were broken off as soon as they appeared. The uninoculated tree was cut back also, but the shoots that grew from its stem were not removed. All of the yellows buds lived and one or more in each tree produced shoots. A total of 11 shoots grew from the 25 yellows buds in the 5 trees having little-peach disease. The shoots were kept under observation over a period of 4 months. During this time they grew

to the following lengths: one to 9 inches, one to 18 inches, two to 20 inches, one to 21 inches, one to 27 inches, two to 28 inches, two to 32 inches, and one to 33 inches. Two of the 5 yellows buds inserted in the healthy tree also produced shoots; one was 14 inches and the other 17 inches long when the experiment was ended. Five shoots grew from the healthy tree. They reached lengths of 13, 18, 23, 25, and 37 inches, respectively. The shoots from the uninoculated healthy tree appeared healthy at all times, as was to be expected. The shoots produced by 2 of the yellows buds inserted in the other healthy tree showed the typical symptoms of yellows as soon as they pushed out. They bore small narrow leaves and numerous upright-growing side branches having short internodes. Yellows was transmitted to this tree. The 11 shoots that grew from the yellows buds inserted in the 5 trees having little-peach disease showed only symptoms of little peach. They bore broad, deep-green leaves of approximately normal size, and produced an undue number of short side branches, as is typical for the little-peach disease. The experiment shows that when yellows buds, which would transmit yellows to healthy trees and would produce shoots bearing typical symptoms of yellows if inserted in healthy trees, are transplanted to trees having little peach, they produce shoots bearing the symptoms of little peach and none of the symptoms of yellows. This suggests that, under the conditions of the experiment, the little-peach virus displaces the yellows virus in the tissues of transplanted buds.

That shoots from yellows buds transplanted to trees having the little-peach disease carry the little-peach and not the yellows virus was shown by subinoculations to healthy trees. Buds from the 11 shoots that grew from yellows buds inserted in the 5 little-peach trees mentioned in the experiment described above were transplanted to the stems of 25 healthy young trees. At the same time a little-peach bud was transplanted to each of 3 similar healthy trees. Three other healthy trees were left uninoculated. Two weeks after this operation, 15 of the 25 test trees were cut back to within 1 inch of the inserted buds. The 3 trees budded with little-peach buds were likewise cut back to within an inch of the inserted buds. The other 10 test trees and the 3 healthy trees were not cut back. As soon as new shoots started to grow from the stems of the cut trees, they were all removed except those from transplanted buds. The buds in the 10 trees that were not cut back did not produce shoots, but they transmitted the little-peach disease to every tree. The 15 shoots from the buds in the test trees that were cut back grew rapidly and bore typical symptoms of little-peach disease. After they had reached a length of 6 or more inches, 1 or 2 other shoots were allowed to grow from the stems of each of the 15 trees. These shoots likewise showed the symptoms of little peach. The shoots produced by these trees were identical in appearance with those derived from the 3 little-peach buds that were

inserted in healthy trees. The experiment proves that subinoculations from shoots produced by yellows buds after they are inserted in little-peach trees transmit the little-peach and not the yellows virus. It also proves that shoots produced by the buds used in making the subinoculations bear only the symptoms of little peach. The subinoculations bring further evidence that the little-peach virus displaces yellows virus in buds transplanted to little-peach trees.

### Inoculation of Trees Having Yellows with Little-peach Virus

When buds from little-peach trees are inserted in healthy trees, they regularly transmit the little-peach disease, but when inserted in yellows trees they invariably fail to transmit this disease. Trees having the yellows disease are fully protected against little peach. In an experiment designed to test the immunity of yellows trees from little-peach disease, 16 healthy young seedlings about 3 feet high and of the same age were selected for uniformity in size and vigor. Ten of the trees were inoculated with yellows by inserting a bud from a yellows tree in the stem of each at a point about 18 inches above the soil level. The other 6 trees were left uninoculated. All of the budded trees came down with yellows within 2 months after their inoculation. Seven months later when the trees were thoroughly diseased, 5 of them were inoculated with the little-peach virus by inserting 4 little-peach buds in the stem of each tree at points varying from 20 to 25 inches above the soil level. Since some transplanted buds fail to develop shoots even when trees are cut back, 4 buds were inserted in each tree in order to secure a fair number of these shoots and also in order to subject the trees to a severe test of immunity from little peach. At the same time 3 of the healthy trees were inoculated with little-peach virus by inserting 4 buds in the stem of each tree at points from 20 to 25 inches above the soil level. The other 3 healthy trees were left uninoculated. Ten days after the insertion of the buds, all trees were cut back to within about 30 inches of the soil level. This was done in order to force out shoots and to promote the growth of inserted buds. All of the transplanted buds lived. The trees were kept under observation during a period of 6 months following the insertion of little-peach buds. The 3 trees inoculated only with little-peach virus came down with little-peach disease in about 6 weeks after the buds were inserted. The 5 yellows trees that were similarly inoculated showed no symptoms of little peach up to the time the experiment was ended. The appearance of these 5 trees was at all times exactly like that of the 5 yellows trees that were not inoculated with little-peach virus. The results are identical with those obtained in several other experiments in which length of time interval between date of inoculation with yellows virus and date of inoculation with little-peach virus was varied. The experiments prove that yellows trees are immune from the little-peach disease.

### Shoots from Little-peach Buds used in the Inoculation of Trees Having Yellows Disease

Seventeen of the 20 little-peach buds that were inserted in the 5 yellows trees mentioned in the experiment described above produced shoots, and 8 of the 12 little-peach buds inserted in the 3 healthy trees mentioned in the same connection likewise produced shoots. The shoots from the little-peach buds in the healthy trees all showed typical symptoms of little peach, but the 17 shoots produced by the little-peach buds that were inserted in yellows trees all showed only symptoms of yellows. Their appearance was exactly like that of the other yellows shoots. However, in order to determine whether, in spite of their appearance, the shoots might carry little-peach virus, subinoculations were made from each of them by budding to a healthy tree. At the same time a bud from each of the 5 trees that had been inoculated only with yellows was inserted in each of the 5 other healthy trees. All of the buds lived, and they all transmitted the yellows disease. No differences were observed in the symptoms shown by the 2 sets of trees. The experiment proves that little-peach buds transplanted to yellows trees not only fail to transmit little peach, but apparently lose the virus of this disease. This suggests that, under the conditions of the experiment, yellows virus is capable of displacing little-peach virus in transplanted buds. Shoots taken from some of the trees used in experiments described above are shown in figure 6. The shoot marked A is from a yellows bud that was transplanted to a healthy tree. The shoot marked C is from a similar yellows bud that was transplanted to a little-peach tree. Shoot A shows the symptoms of yellows, while C bears the symptoms of little peach. Shoot B grew from a little-peach bud that was transplanted to a healthy tree, while shoot D grew from a similar bud transplanted to a yellows tree. Shoot B bears typical symptoms of little peach, while shoot D shows the symptoms of yellows. The symptoms of the 2 diseases are not satisfactorily portrayed by the pictures because they do not bring out color differences, but it is easy to see that shoot A looks like shoot D and that shoot B looks like shoot C. The waviness shown by some of the leaves on shoot C is due to the fact that this shoot wilted slightly before the picture was made.

### Further Evidence of Displacement of One Virus by Another

The fact that yellows buds transplanted to little-peach trees produce shoots that bear the symptoms of little peach, and that little-peach buds transplanted to yellows trees produce shoots that bear the symptoms of yellows, indicates that, under suitable conditions, either virus is capable of displacing the other in infected tissues. Since, however, new shoots are produced from cells in the growing points of the buds and there is at present no way of determining whether or not these cells are infected at the time the

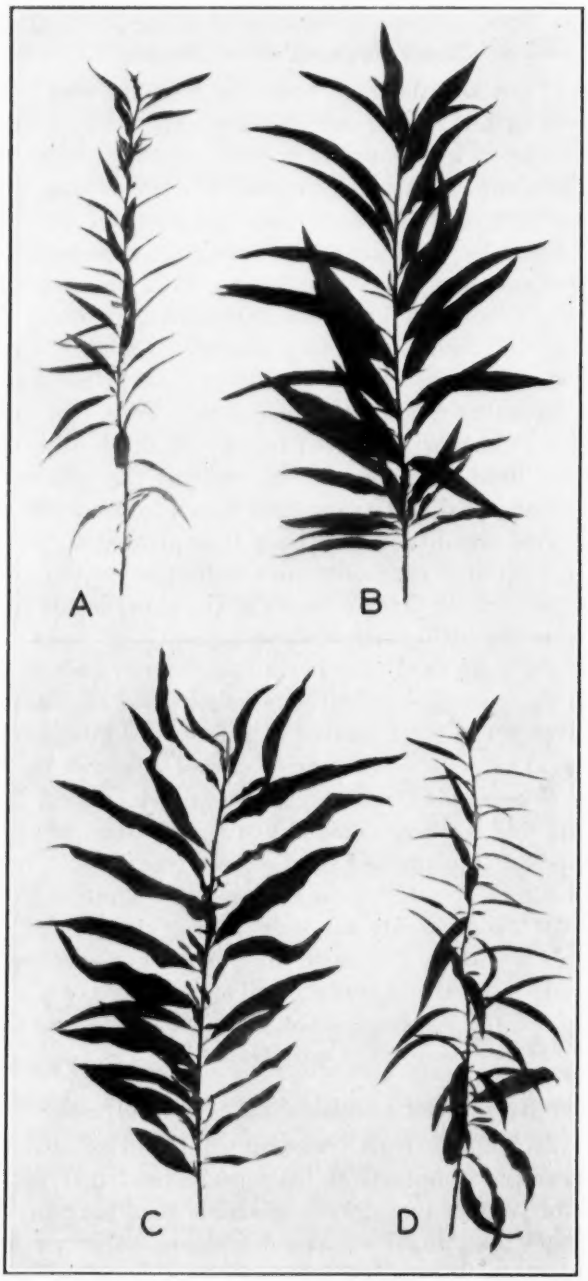


FIG. 6. Four shoots that grew from transplanted buds. Shoots A and C are from yellows buds that grew in healthy and in little-peach trees, respectively, while shoots B and D are from little-peach buds that grew in healthy and in yellows trees, respectively. Shoots C and D show the symptoms of the diseases carried by the trees in which they grew rather than the symptoms of the diseases carried by the buds that produced them.

buds are transplanted, it seemed possible that virus from the trees in which the buds were inserted might move into the new growth without displacing the other virus in cells of the older tissues of the buds. In order to obtain evidence on this point, 3 yellows buds that had been transplanted to little-peach trees and had grown in these trees for a period of 11 months without producing shoots, and 3 little-peach buds that had grown in yellows trees for the same period and had likewise produced no shoots, were carefully removed. Their edges were trimmed sufficiently to insure that no bark from the trees on which they had grown would adhere to the buds. One bud was then inserted in each of 6 healthy trees. The object of the test was to determine whether any given bud would transmit yellows virus, little-peach virus, or a mixture of the 2 viruses. The trees were under observation during a period of 4 months following their inoculation. The 3 inoculated with little-peach buds developed marked symptoms of yellows, and the 3 inoculated with yellows buds developed equally marked symptoms of little peach. There was no symptomatological evidence of a mixture of the 2 viruses in any of the 6 trees. The experiment proves that a yellows bud, that would transmit yellows to any healthy tree into which it might be inserted, will transmit the little-peach and not the yellows virus if it is transplanted and allowed to grow in a little-peach tree for a period of 11 months previous to its insertion in the healthy tree, and, likewise, that a little-peach bud capable of transmitting the little-peach virus to any healthy tree will transmit the yellows virus and not the little-peach virus if transplanted and left to grow in a yellows tree for the same period of time previous to its insertion in the healthy tree. These results support the view that, under suitable conditions, either of the viruses may displace the other, even in old tissues that are thoroughly invaded. It is, of course, possible that in removing the buds from the diseased trees into which they had been transplanted, a few cells from these trees may have adhered to the inner surfaces of the buds, but this seems unlikely since microscopic studies on stained sections through transplanted buds show that new cambium regularly develops from the bark side of the union and not from the stem tissues to which the transplanted buds adhere.

### Dominance of One Disease over the Other Determined by Point of Inoculation

When it was found that yellows trees are immune from little peach, and that little-peach trees are immune from yellows, experiments were undertaken for the purpose of determining whether mixed infections could be obtained by the simultaneous inoculation of healthy trees with the 2 viruses. Seedlings were inoculated by inserting a little-peach bud and a yellows bud in their stems at convenient distances above the soil level. One bud was usually inserted about 12 inches and the other from 13 to 15 inches above

the surface of the soil. In some instances the yellows bud was placed at the 12-inch level and the little-peach bud at a higher level, while in other cases the position of the 2 buds was reversed. These experiments yielded unexpected results. The trees always came down with the disease carried by the bud placed in the uppermost position. When the yellows bud was inserted at the 15-inch level and the little-peach bud at the 12-inch level, the trees always came down with the yellows disease, but when the position of the buds was reversed they always came down with the little-peach disease. One or both of the transplanted buds frequently produced shoots. Any shoot that grew from a bud in the uppermost position always showed the symptoms of the disease carried by that bud, but any shoot that grew from a bud in the lower position showed the symptoms of the disease carried by the upper bud and no symptoms of the disease carried by the bud from which the shoot grew. In one of these experiments, 12 healthy young trees were inoculated with both the yellows and little-peach viruses. The yellows buds were inserted above the little-peach buds in 6 of the trees and below the little-peach buds in the other 6 trees. The lower buds were placed about 12 inches and the upper buds about 14 inches above the soil level. Six similar trees served as controls. Two of these were inoculated with little-peach virus only, 2 with yellows virus only, and 2 were left uninoculated. The trees were kept under observation during a period of 15 months following inoculation. All of the buds lived. The 6 trees in which yellows buds were inserted above little-peach buds came down with yellows within 2½ months after their inoculation. The 6 trees in which yellows buds were inserted below little-peach buds all came down with little peach. The 2 control trees inoculated only with yellows virus and the 2 inoculated only with little-peach virus came down with yellows and with little peach, respectively, within about the same period of time. Seven of the 12 buds placed at the lower level in the 12 test trees produced shoots varying from 12 to 20 inches in length. Four of these carried the little-peach virus and 3 carried the yellows virus when they were inserted. The 4 that carried the little-peach virus produced shoots bearing symptoms of yellows, and the 3 that carried the yellows virus produced shoots bearing the symptoms of little peach. Subinoculations from the shoots with yellows symptoms resulted in the transmission of the yellows disease, while subinoculations from shoots with little-peach symptoms gave the little-peach disease.

The experiment proves that point of inoculation determines which virus will prevail in any given tree to which both are transferred on the same date. Here again the results suggest that either virus is capable of displacing the other. Results similar to those reported above were obtained even when the 2 buds were not inserted on the same date. When a bud was transplanted to the upper position either a few days before or a few days after that placed in the lower position, the trees always came down with the disease carried by the bud

in the upper position. When, however, the interval between the dates on which the 2 buds were inserted was as much as 3 weeks, the trees frequently showed symptoms of both diseases, provided the buds placed in the lower position were inserted first. In such cases the disease carried by the upper buds affected the upper parts of the trees, while that carried by the lower buds affected the lower parts of the trees. It seems that the virus carried by the upper bud does not prevail if that carried by the lower bud is sufficiently well established in the base of the tree when the other bud is inserted in the upper position.

## DISCUSSION

The inoculation of yellows trees and little-peach trees with rosette virus always causes infection and the virus spreads with about the same rapidity in such trees as in healthy ones. Moreover, there is no evidence that the rosette disease loses any of its severity in either yellows trees or little-peach trees. In these respects the behavior of the rosette virus when inoculated into trees affected by yellows or little peach appears to stand in sharp contrast to the behavior of yellows virus when inoculated into trees affected by little peach or the behavior of little-peach virus when inoculated into trees affected by yellows. The immunity tests bring no evidence of a close relationship between rosette and either of the other diseases.

Since yellows trees are immune from little peach and little-peach trees are immune from yellows, it is concluded that these 2 diseases are closely related, and that little peach should be classified as a mild strain of yellows. The fact that little peach appeared about 100 years later than yellows would suggest that it may have arisen as a variant of the latter.

It is interesting to note that the immunity reactions shown by the peach indicate a close relationship between two virus diseases that can not be mechanically transmitted except by tissue transplantation. Up to the present time the serological technique has not been successfully employed in demonstrating relationships between such diseases. Plant immunity tests are, therefore, especially valuable in taxonomic studies on diseases of this type.

The immunity reactions described above can not be used at present in controlling either little peach or yellows, because, although little peach is less severe than yellows, both ruin affected trees. Practical use of immunity reactions must await the finding of a strain of virus so mild as to cause no serious damage to either trees or fruits.

The most surprising result of the cross-immunity tests with little-peach and yellows viruses is the observation that either may apparently displace the other from infected buds. The fact that shoots bearing yellows symptoms are produced by little-peach buds inserted in yellows trees, and shoots bearing little-peach symptoms by yellows buds transplanted to little-peach trees, brings evidence that this occurs. The same phenomenon was ob-

served in trees inoculated with both viruses simultaneously by means of buds placed at different levels. When yellows virus is carried by the bud in the upper position the dominance of yellows symptoms might be explained by assuming that the severe disease, yellows, obscures the symptoms of the mild disease, little peach, but such an explanation becomes inadequate when the position of the buds is reversed and the mild disease caused by little-peach virus prevails over the severe disease caused by yellows virus. That a tree infected locally by a virus that would cause it to become severely diseased may be made to come down with a less severe disease by a subsequent inoculation with virus of the latter is a striking fact. Since shoots produced by the lower buds always bear symptoms of the disease carried by the upper buds, it is believed that the location of the points at which the two viruses are introduced determines in some manner which virus will displace the other. But the means by which these displacements are accomplished are not yet known. It is possible that the two viruses use the same materials for multiplication, and that the one present in the large member of the union (the tree) overgrows the one present in the small member (the transplanted bud). How the virus in a bud inserted at one level in a tree can replace the virus in another bud inserted at a lower level in the same tree is not explained by any theory involving mass action, for approximately equal doses of the viruses are used in making inoculations. Köhler (2) has reported that when tobacco plants infected with a weak strain of the Y-virus of potato are inoculated with a strong strain of the same virus, the latter displaces the former in the leaves at the top of the plant, and he has suggested that the weak virus is assimilated by the strong one (". . . dass das schwache Virus von den starken vollständig unterdrückt, vielleicht sogar assimiliert wird.").

Both the little-peach and the yellows (3) virus move much more rapidly downward than upward in newly infected trees. It might be expected that virus in a bud inserted low down on the stem of a tree rather than virus in a bud inserted at a higher level would invade the lower portions of the tree. There is at present no explanation as to why the reverse occurs. It is conceivable that virus from the lower bud does invade the tree, and that it is not completely displaced by virus from the upper bud; but if this occurs the virus from the lower bud is present without producing symptoms that can be detected in young trees. In any case, the virus in the upper bud dominates.

It should be clearly understood that the experiments described in this paper do not prove that little-peach virus fails to invade yellows trees when little-peach buds are inserted in the trees, or that yellows virus fails to invade little-peach trees when yellows buds are inserted in these trees. All that can be said is that, if such invasions have occurred, they have not caused detectable modifications in the symptoms shown by the trees. Each disease protects against the other.

SUMMARY AND CONCLUSIONS

1. A description is given of the symptoms produced in young peach seedlings by the rosette, yellows, and little-peach viruses.

2. Rosette virus readily invades trees affected by either little peach or yellows and neither of these diseases gives any protection against rosette. Therefore, it is concluded that rosette is not closely related to little peach or yellows.

3. Trees having little-peach disease are immune from yellows, and trees having yellows are immune from little peach. Since invasion of trees by either virus protects them against the disease caused by the other, it is concluded that little peach and yellows are closely related and should be classified as strains of the same disease.

4. Yellows buds transplanted to little-peach trees produce shoots that show typical symptoms of little-peach disease, and little-peach buds transplanted to yellows trees produce shoots that show typical symptoms of yellows. Subinoculations from these shoots transmit the disease carried by the tree into which the bud was transplanted and not the disease carried by the bud at the time it was transplanted. Therefore, it is concluded that either virus may displace the other in infected tissues.

5. Trees inoculated simultaneously with both the little-peach and the yellows virus by means of buds inserted at different levels in their stems come down with the disease carried by the bud in the upper position. Any shoot produced by the bud in the lower position will bear the symptoms of the disease carried by the bud in the upper position. Subinoculations from such a shoot transmit the disease carried by the bud in the upper position. It is concluded that point of inoculation determines which virus will prevail in any given tree.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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# EFFECT OF CROWN RUST ON THE COMPOSITION OF OATS[1]

H. C. MURPHY[2]

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## INTRODUCTION

The effect of crown rust (*Puccinia coronata avenae* Eriks.) on the yield and water economy of oats has been reported recently by the writer (7).[3] The grain harvested in these earlier studies afforded excellent material for use in determining the effect of crown rust on the composition of the grain of varieties initially infected at different stages of development. Additional studies were conducted to determine the effect of crown rust on the yield and composition of green plants of susceptible and resistant varieties. Long (4) observed in 1919 that oat leaves, heavily infected with crown rust, contained 6.82 per cent of reducing substances, while rust-free leaves contained 15.24 per cent. Caldwell *et al.* (3) have made a rather complete study of the effect of leaf rust (*P. triticina* Eriks.) on the composition of wheat plants and grain. Otherwise, there is little published information regarding the effect of the various cereal rusts on the composition of their hosts. A preliminary report of the data presented herein has been published by the writer (6).

## METHOD AND MATERIALS

In the spring of 1930, pure-line selections of Victoria (C.I.[4] 2401) and Markton (C.I. 2053) were grown in the greenhouse in soil of uniform moisture and artificially inoculated with physiologic form 1 of crown rust at different stages of development. There were 50 1-gal. jars, each containing 4 plants of the Victoria selection and another set of equal numbers containing Markton plants. The plants in 10 jars of each set were dusted with an abundance of urediospores of crown rust when they were in the seedling (4-leaf) stage. A like number of each set was dusted in the early boot, early

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[3] Reference is made by number to Literature Cited, p. 234.

[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases.

anthesis, and early dough stages, respectively, and each of the 4 lots was reinoculated every two weeks. The water requirement and the yield of grain, straw, and roots of these plants, as well as a more detailed description of the technique involved, have beeen published (7).

The grain harvested from the plants studied in 1930 was retained. In March, 1934, a sufficient quantity of this grain was carefully hulled by hand to allow 35 grams of kernels of each lot. The samples of kernels and hulls were sent to H. J. Brownlee of the Science Department, Quaker Oats Company, Cedar Rapids, Iowa, who kindly determined the 6 crude constituents: moisture, ash, protein, fat, fiber, and nitrogen-free extract.

On October 6, 1933, pure-line selections of the varieties Markton (C.I. 2053), Iogold (C.I. 2329), Victoria (C.I. 2401), and Bond (C.I. 2733) were planted uniformly in 1-gal. jars, with 6 jars for each selection. After emergence, the number of plants was reduced to 15 per jar. On December 22, 1933, the plants in half of the jars of each selection were inoculated uniformly in a manner similar to that used in 1930. The susceptible selections showed 100 per cent infection within 10 days. The same plants were reinoculated on January 5, 1934.

On January 19, 4 weeks after the first inoculation, samples of the infected and rust-free plants of each selection were taken for chemical analyses. At that time the rust-free plants were all in the boot stage and the Iogold plants were just at the point of showing first heading. All of the plants in the 3 jars of each lot were cut close to the soil line and the total green weight from each jar was recorded. Representative duplicate 100-gram samples were immediately cut into small pieces and dropped into boiling alcohol. Additional duplicates samples were obtained for moisture determination. The 100-gram samples were extracted with 80 per cent alcohol and the extract was made to standard volume. From this fraction, aliquot portions were taken for determination of alcohol-soluble solids, ash in extract, alcohol-soluble nitrogen, and sugars. After extraction the residue was oven-dried, weighed, and recorded as the alcohol-insoluble fraction. Portions of the residue were used for the determination of ash, alcohol-insoluble or residual nitrogen, and the polysaccharides. Moisture was determined by drying the green samples, taken for that purpose, to a constant weight in an electric oven at a temperature of 100° C. Soluble solids were determined by drying aliquot portions of the alcoholic extract in a vacuum oven at a temperature of 75° C., and total solids were obtained by adding the values of the soluble and insoluble fractions. Ash was determined by igniting the soluble and insoluble solids in an electric oven at a dull red heat until constant weight was obtained. The nitrogen of the residue was determined by the Kjeldahl method, and that of the extract by the reduced iron-powder method described by Pucher *et al.* (8). The value of the total

nitrogen was obtained by adding these two values. Ammonia, amide, and nitrate and nitrite nitrogens were determined by the methods described by Appleman *et al.* (1). Sucrose and glucose were determined by the volumetric permanganate Munson-Walker method, levulose by the Jackson method. An attempt was made to digest starch from the residue after the dextrin had been previously extracted with 10 per cent alcohol and hydrolyzed with hydrochloric acid. The acid-hydrolyzable polysaccharides in the residue from the starch determination were determined by boiling with hydrochloric acid.

All analytical data reported in this paper have been calculated on the air-dry-weight basis for the grain, and on the green- and dry-weight bases for the green plants. Unless otherwise stated, the methods of analysis used were those described in Methods of the Association of Official Agricultural Chemists (2). The yields recorded for the green-plant material represent in each case the average of 3 jars. The percentages of the different chemical constituents each represent the average of duplicate samples.

## EFFECT OF CROWN RUST ON COMPOSITION OF GRAIN

The susceptible Markton plants initially infected in the seedling and boot stages failed to produce grain and thus prevented any study of the effect of early infection on the composition of the grain of very susceptible plants. Markton plants initially inoculated in the anthesis stage produced 54. 7 per cent as much grain as rust-free plants, while the yield of resistant Victoria plants initially inoculated in the seedling stage was 52.5 per cent of that of the rust-free ones. Crown-rust infection brought about a marked decrease in the yield of grain and an increase in the percentage of hulls, on both susceptible and resistant varieties, although the effect was much greater on the susceptible one. The results of this phase of the study have been presented in detail in a previous publication (7).

The outstanding finding of the present phase of this investigation is that, although crown rust had a very pronounced effect on the yield of grain and the relative percentage of kernels and hulls, it had little effect on the crude chemical composition of either kernels or hulls (table 1). Other than for the crude protein and nitrogen-free-extract contents of the kernels, there appeared to be no consistent relationship between rust infection and composition of either kernels or hulls. All of the other differences were small, inconsistent, and probably not significant. There was, however, a consistent, although slight, increase in the percentage of crude protein and decrease in the percentage of nitrogen-free extract of the kernels in relation to earliness of infection, *i.e.*, the earlier infection appeared, the greater was the increase in proportion of protein and the decrease in proportion of nitrogen-free extract. These relationships hold for both the susceptible and

resistant varieties, although the differences, particularly for the nitrogen-free extract, are very small and the apparent relationship might be a result of chance arrangement. Duration of infection had more effect on the crude-protein and nitrogen-free-extract content of the kernels than did type of infection. Markton, initially infected during anthesis, showed an increase in crude protein content of the kernels of 4.3 per cent and a decrease in nitrogen-free-extract content of 0.3 per cent, while Victoria plants initially infected at the same time showed an increase and a decrease of 5.1 and 1.9 per cent, respectively. Victoria plants initially inoculated in the seedling stage showed an increase in crude protein content of the kernels of 12.1 per cent and a decrease in nitrogen-free extract of 3.6 per cent.

### EFFECT OF CROWN RUST ON COMPOSITION OF GREEN PLANTS

The writer (7) has shown that crown rust materially reduces the yield of oat straw, although the reduction is not so great as that for the grain or roots. Oats often are harvested before maturity and used as a soiling crop or as a hay crop. In addition to the study of the effect of crown-rust infection on the yield and composition of the grain a study was made to determine the effect on yield and composition of the green plant. Because of the great reduction in yield of the susceptible varieties resulting from the rust infection, all the plants were harvested while these heavily infected plants were still green and in the boot stage of development. If harvest had been delayed until the milk stage, as was originally planned, the infected susceptible plants probably would have been dead. Under field conditions infections occur often as early in the plant's stage of development as that initiated in this experiment and the effect produced by the rust at this stage probably is as important as that at a later stage.

Markton, Iogold, Victoria, and Bond were used because they range in reaction from the completely susceptible Markton to the nearly immune Bond (5). Urediospores of physiologic form 1 were used for all inoculations. Markton and Iogold each showed an equal percentage of infection amounting to 100 per cent, while the amount of infection on Victoria and Bond was approximately 85 and 40 per cent, respectively. Markton was completely susceptible, showing large uredia with very slight chlorotic areas adjoining them. Iogold was slightly less susceptible than Markton, showing somewhat smaller uredia with more adjoining chlorosis. Victoria was very resistant, showing few minute uredia with heavy necrotic areas surrounding them, and much necrotic area without uredia. Bond was nearly immune, showing necrotic and chlorotic flecks only.

From a physiological standpoint the effect of the rust on the green plant is best shown by calculating the percentage of the various constituents on a green-weight basis, as they actually occurred in the living plant; while from

TABLE 1.—*Effect of crown rust on the chemical composition of grain of oat varieties initially infected at different stages of development*

| Variety | Stage at initial infection | Yield[a] | Portion of total grain | Moisture | Ash | Crude protein (N × 6.25) | Fat | Fiber | Nitrogen-free extract |
|---|---|---|---|---|---|---|---|---|---|
| | | *Grams* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| | | | | | Kernels | | | | |
| Markton (Susceptible) | Anthesis | 14.1 ± .5 | 68.6 | 6.1 | 2.3 | 16.9 | 6.7 | 2.3 | 65.7 |
| | Control | 25.8 ± .1 | 79.4 | 6.0 | 2.4 | 16.2 | 6.9 | 2.6 | 65.9 |
| Victoria (Resistant) | Seedling | 12.6 ± .2 | 70.8 | 5.9 | 2.4 | 22.2 | 6.1 | 2.6 | 60.8 |
| | Boot | 16.6 ± .4 | 72.1 | 5.5 | 2.5 | 21.5 | 6.3 | 2.4 | 61.8 |
| | Anthesis | 19.6 ± .4 | 73.9 | 6.1 | 2.4 | 20.8 | 6.3 | 2.5 | 61.9 |
| | Control | 24.0 ± .2 | 75.0 | 6.3 | 2.2 | 19.8 | 6.4 | 2.2 | 63.1 |
| | | | | | Hulls | | | | |
| Markton (Susceptible) | Anthesis | ..... | 31.4 | 3.6 | 7.1 | 1.8 | 0.8 | 34.4 | 52.3 |
| | Control | ..... | 20.6 | 3.9 | 7.3 | 2.6 | 0.7 | 31.7 | 53.8 |
| Victoria (Resistant) | Seedling | ..... | 29.2 | 3.1 | 7.4 | 4.2 | 0.8 | 31.4 | 53.1 |
| | Boot | ..... | 27.9 | 4.5 | 7.5 | 3.2 | 0.9 | 33.8 | 50.1 |
| | Anthesis | ..... | 26.1 | 4.1 | 7.1 | 3.0 | 0.4 | 32.8 | 52.6 |
| | Control | ..... | 25.0 | 4.4 | 7.2 | 3.4 | 0.8 | 31.9 | 52.3 |

[a] Average yield of grain (kernels plus hulls) per jar, of 10 jars with four plants each.

a practical or feeding standpoint it is preferable to present the relative proportions of certain of the general constituents calculated on a dry-weight basis.

### Yield

The average yields (green weight) of the infected Markton, Iogold, Victoria, and Bond plants were decreased 69.3, 63.6, 22.2, and 14.7 per cent, respectively, as a result of crown-rust infection (Table 2 and Figs. 1 and 2). The yield of these plants, expressed as dry weight, is shown in table 3. The amount of change calculated on a dry-weight basis is very similar to that obtained on a green-weight basis because there is little variation in the moisture content between the different samples. The relative susceptibility of each variety was reflected by the decrease in yield of the infected plants. It is obvious, however, that the differences between the effect on the resistant and susceptible varieties would be greater under natural field conditions because of the partial or complete inability of the pathogen to reproduce on the more resistant plants, consequently limiting the supply of inoculum available for secondary and later infections.

### Moisture

The infected susceptible plants contained slightly less moisture than the rust-free ones, while the moisture content of the infected resistant plants was slightly higher than that of the rust-free plants. The differences are very slight and, although consistent, they are of doubtful significance. It is worthy of note, however, that if the percentage of moisture is ascertained in an entirely different manner, *i.e.*, by subtracting the total solids from the green weight, the same relationship exists and the comparative percentages thus obtained are apparently well within the range of experimental error.

### Solids

Crown rust brought about a definite decrease in the percentage of alcohol-soluble solids in the plant. This decrease ranged from 19.3 per cent for Markton to 10.8 per cent for Bond. Whereas the soluble solids were decreased as a result of crown-rust infection, the proportion of alcohol-insoluble solids was increased in amounts ranging from 29.6 to 4.0 per cent for Markton and Bond, respectively. The total solids were slightly increased in the 2 susceptible varieties as a result of infection but decreased in the resistant Victoria and Bond varieties. The decrease in soluble and the increase in insoluble solids is, in part at least, a direct result of the effect of the rust on the percentage of nitrogen, sugars, and polysaccharides in the plants, as shown later.

### Ash

Plants infected with crown rust showed regularly a greater total ash content than rust-free plants. The percentage of ash in both the extract

TABLE 2.—*Effect of crown rust on yield and composition of green plants of susceptible and resistant oat varieties (green-weight basis)*

| Variety | Condition of plants | Yield | Moisture | Solids | | Ash | | Nitrogen | | | | | Sugars | | | Polysaccharides | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Total soluble | Total insoluble | Total soluble | Total insoluble | Ammonia | Amide | Nitrate and nitrite | Total soluble | Total insoluble | Sucrose | Glucose | Lævulose | Dextrin | Acid hydrolyzable |
| | | *Grams* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Markton (Susceptible) | Infected | 81.07 | 81.33 | 5.62 | 13.52 | 1.54 | 1.26 | .050 | .047 | .211 | .319 | .297 | .40 | .40 | .03 | .56 | 2.75 |
| | Rust-free | 264.08 | 82.29 | 6.96 | 10.43 | .81 | .67 | .014 | .011 | .050 | .084 | .236 | 2.44 | 1.88 | 1.16 | .73 | 2.12 |
| Iogold (Susceptible) | Infected | 89.96 | 83.15 | 5.25 | 11.42 | 1.04 | 1.07 | .039 | .039 | .230 | .316 | .254 | .42 | .35 | .06 | .64 | 2.36 |
| | Rust-free | 247.14 | 83.67 | 6.32 | 10.08 | .76 | .82 | .011 | .013 | .055 | .084 | .224 | 2.55 | 1.74 | 1.13 | .81 | 1.97 |
| Victoria (Resistant) | Infected | 172.05 | 83.31 | 5.37 | 10.74 | 1.56 | .90 | .013 | .028 | .050 | .185 | .288 | .95 | .45 | .39 | .50 | 1.99 |
| | Rust-free | 221.10 | 82.77 | 6.53 | 10.13 | 1.30 | .74 | .011 | .010 | .033 | .088 | .270 | 2.37 | 1.34 | 1.14 | .60 | 1.90 |
| Bond (Nearly immune) | Infected | 235.97 | 85.03 | 5.70 | 9.20 | 1.03 | .57 | .011 | .014 | .026 | .105 | .248 | 2.54 | 1.41 | .79 | .61 | 1.87 |
| | Rust-free | 276.50 | 84.70 | 6.39 | 8.85 | .94 | .53 | .011 | .010 | .025 | .067 | .239 | 3.15 | 2.02 | 1.17 | .70 | 1.81 |

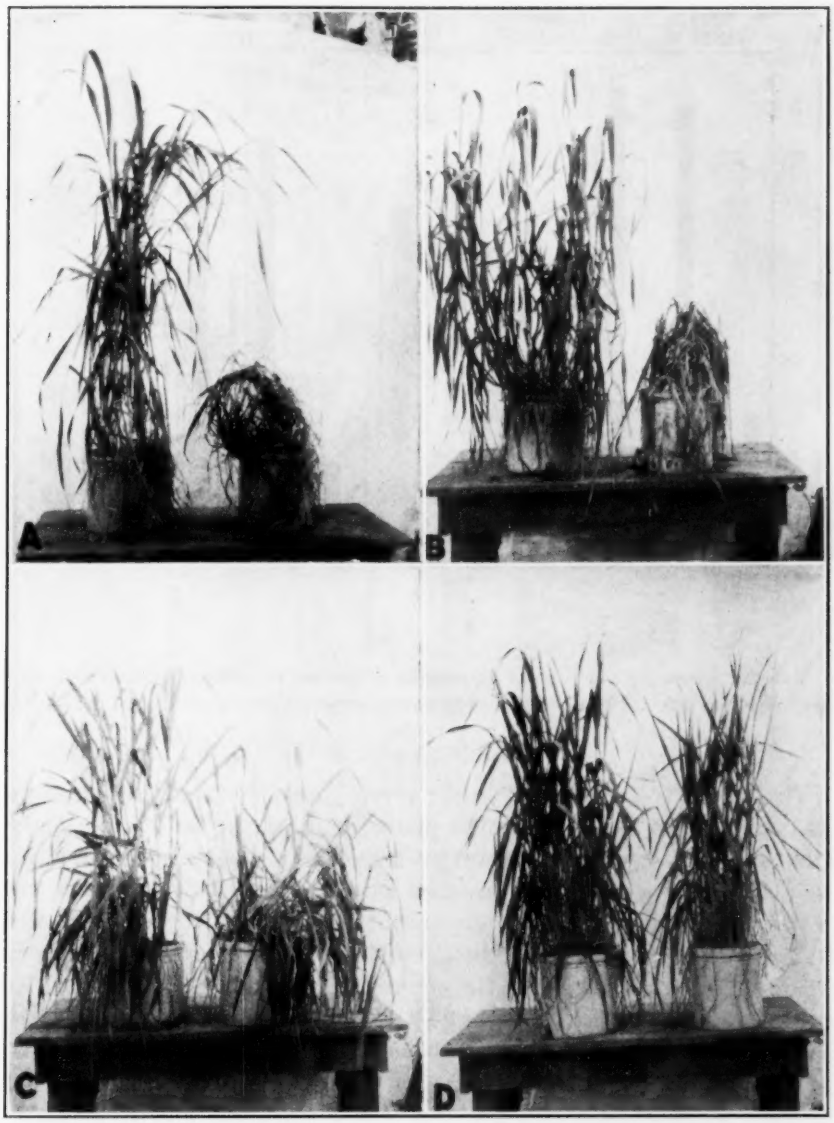


FIG. 1. A. Rust-free (left) and infected (right) plants of Markton. B. Iogold. C. Victoria. D. Bond.

and residue was increased as a result of the rust infection. Markton showed the greatest increases both in alcohol-soluble and alcohol-insoluble ash content, amounting to 90.1 and 88.0 per cent, respectively, while for Bond the increases were 9.6 and 7.5 per cent, respectively.

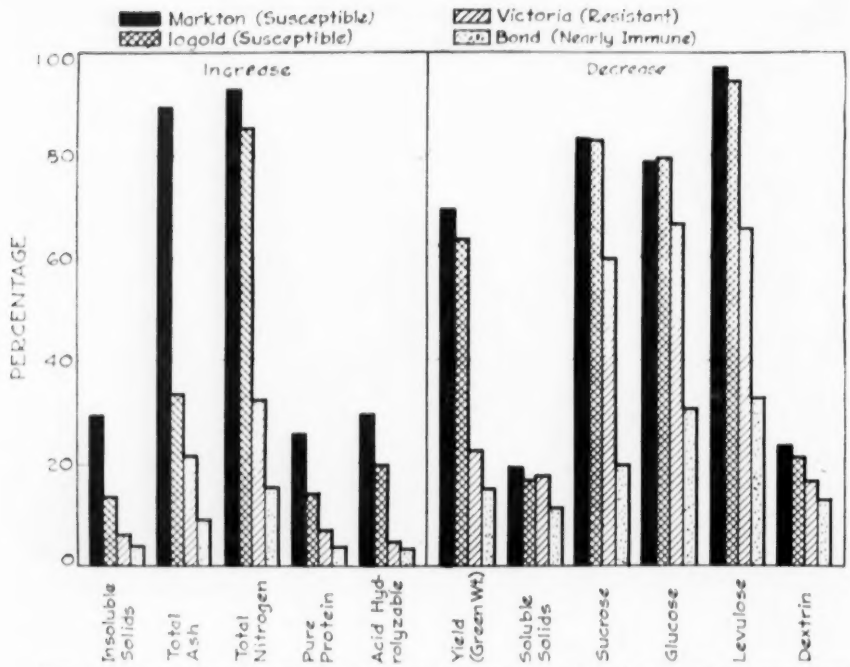


FIG. 2. Percentage increase or decrease in proportion of certain chemical constituents and of yield for infected green plants of Markton, Iogold, Victoria, and Bond oat varieties.

## Nitrogen

The total nitrogen content in the green plants was small, amounting to less than one per cent. Infected plants, both of the susceptible and resistant varieties, contained a higher proportion of alcohol-soluble and alcohol-insoluble nitrogen than did rust-free plants. The difference was much greater for the soluble or noncolloidal nitrogen, amounting to 279.8, 276.2, 110.2 and 56.7 per cent, respectively, for the infected Markton, Iogold, Victoria, and Bond plants, while the corresponding increase in proportion of insoluble or colloidal nitrogen was 25.8, 13.4, 6.7, and 3.8 per cent. Striking increases in the percentages of ammonia, amide, and nitrate and nitrite nitrogens accounted for most of the increase in the noncolloidal nitrogen. The percentage of ammonia nitrogen was 257.1, 254.5 and 18.2 per cent greater in the infected plants of Markton, Iogold, and Victoria, respectively, than in the rust-free plants, while for the nearly immune Bond there was no difference. Amide nitrogen was increased 327.3, 200.0, 180.0, and 40.0 per cent, respectively, in the infected plants of these same varieties, while the corresponding increase in nitrate and nitrite nitrogen was 322.0, 318.2, 51.5, and 4.0 per cent. The percentage of nitrate and nitrite nitrogen was greater than that of the ammonia and amide nitrogen combined.

TABLE 3.—*Effect of crown rust on yield and composition of green plants of susceptible and resistant oat varieties (dry-weight basis)*

| Variety | Condition of plants | Yield | Total ash | Total nitrogen | Total crude protein[a] | Alcohol insoluble protein[b] | Total sugars | Total polysaccharides | Total carbohydrates |
|---|---|---|---|---|---|---|---|---|---|
| | | *Grams* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Markton | Infected | 15.14 | 15.00 | 3.30 | 20.63 | 9.94 | 4.45 | 17.73 | 22.18 |
| (Susceptible) | Rust-free | 46.77 | 8.36 | 1.81 | 11.31 | 8.33 | 30.94 | 16.09 | 47.03 |
| Iogold | Infected | 15.16 | 12.52 | 3.38 | 21.13 | 9.42 | 4.93 | 17.80 | 22.73 |
| (Susceptible) | Rust-free | 40.36 | 9.68 | 1.89 | 11.81 | 8.57 | 33.19 | 17.02 | 50.21 |
| Victoria | Infected | 28.72 | 14.74 | 2.83 | 17.69 | 10.87 | 10.72 | 14.92 | 25.64 |
| (Resistant) | Rust-free | 38.10 | 11.84 | 2.08 | 13.00 | 9.80 | 28.21 | 14.51 | 42.74 |
| Bond | Infected | 35.32 | 10.69 | 2.36 | 14.75 | 10.35 | 31.66 | 16.57 | 48.23 |
| (Nearly immune) | Rust-free | 42.30 | 9.61 | 2.00 | 12.50 | 9.76 | 41.44 | 16.40 | 57.84 |

[a] Total nitrogen × 6.25.

[b] Insoluble nitrogen × 6.25.

From the standpoint of feeding value the increase in the ammonia, amide, and nitrate and nitrite nitrogen are not necessarily beneficial; in fact, they are more likely detrimental. On the other hand, the smaller increase in the colloidal or protein nitrogen in itself doubtless increases the feeding value of the infected plants. This apparent increase is, however, offset many times by the decrease in sugars and dextrin, not to mention the reduced yield of the infected plants.

The effect of crown-rust infection on the percentage content of total crude and alcohol-insoluble protein is shown in table 3. Although the total crude protein (total nitrogen $\times$ 6.25) is almost doubled in the diseased Markton and Iogold plants, this is obviously not an accurate estimation of the actual protein content of the plants because of the large proportion of inorganic and non-protein nitrogen present in the extract, especially from the diseased plants. If we consider alcohol-insoluble or colloidal nitrogen only and calculate what might be termed "pure protein" by multiplying this amount by the factor 6.25, then the greatest increase in protein content is 19.3 per cent, in the infected Markton plants.

Since the rust mycelium and spores that still adhered to the plant were analyzed along with the host tissue, it is probable that the additional colloidal nitrogen or "pure protein" found in the infected plants was present mainly in the fungus. At least, the ratio of increase in the insoluble nitrogen (Table 2) appears to be in close relation to the relative susceptibility of the different varieties and thus to vary directly with mycelial development. On the other hand, it would appear more likely that the great increase in inorganic nitrogen was caused by the fungus interfering with metabolism and translocation.

## Sugars

Of the total sugars determined in the rust-free oat plants, approximately one-half was sucrose, slightly more than one-fourth, glucose, and slightly less than one-fourth was levulose (Table 2). The effect of crown-rust infection on the percentages of these sugars in the susceptible plants was striking and in direct contrast to the effect on the ash and nitrogen content. The decrease in percentage content of sucrose, glucose, and levulose in Markton, as a result of rust infection, was 83.6, 78.7, and 97.4 per cent, respectively, while the corresponding decrease in Iogold was 83.5, 79.9, and 94.7 per cent. These two varieties were very susceptible, and, in addition to the infected plants containing considerable amounts of mycelium and adhering spores, additional quantities of mature urediospores were lost. From these studies there is no certain means of knowing whether the lower percentage of sugars in the infected plants was caused by increased respiration of the host, consumption by the fungus, or interference with photo-

synthesis caused by shading, mechanical obstruction, etc. Probably all of these factors were involved to some extent.

Very few spores were produced by the fungus on the resistant Victoria variety. Microscopic examination of cross sections of infected leaves also revealed much less mycelial growth in Victoria leaves than was found in the two susceptible varieties, although necrotic areas covered most of the leaf surface. Reductions in percentages of sugars in infected Victoria plants, ranging from 59.9 per cent for sucrose to 66.4 per cent for glucose, show that infection characterized by heavy necrosis has a pronounced effect on sugar content.

Considering the difference in percentage of infection on the varieties (Markton and Iogold 100 per cent, Victoria 80 per cent) it appears that there is a close relationship between the percentage of infection and the reduction of the various sugars. This would seem to indicate that the main factor affecting the amounts of these sugars is interference with photosynthesis, such as shading and destruction of chlorophyl. When different infection types are involved, percentage of infection does not measure the relative amounts of mycelium or spores present. The infection on Bond was characterized by chlorotic flecks and amounted to approximately 40 per cent of the leaf area. Apparently the reduction in sugars was about proportional to the percentage of infection as determined by these chlorotic areas. There was, however, very little if any mycelial development in these infected leaves and no spores. Absence of chlorophyl apparently accounted for the reduced amount of the sugars. The apparent correlation between total sugars and percentage of infection on the 4 varieties is also quite evident (Table 3).

### Starch

No appreciable quantity of starch was found in either the rust-free or infected plants of any of the 4 varieties studied. Starch is a relatively unimportant constituent of the green oat plant.

### Dextrin

The proportion of dextrin in the plants studied did not exceed 0.81 per cent. Crown-rust infection decreased it in amounts ranging from 23.3 per cent for Markton to 12.9 per cent for Bond.

### Acid hydrolyzable

In contrast to the effect on dextrin, crown-rust infection brought about an increase in percentage of acid-hydrolyzable substances. This is in agreement with the increase in insoluble solids and probably represents an increased balance of pectic and hemicellulose materials as a result of the consumption or retardation in the formation of sugars, dextrin, etc., by the

fungus. The ratio of the amounts of reduction in these substances for the different varieties was dependent apparently upon their relative susceptibility as measured by both percentage and type of infection.

## DISCUSSION

The data reported herein and in a previous publication (7) present a fairly complete picture of the effect of crown-rust infection on the yield, water requirement, and composition of susceptible and resistant oat varieties under greenhouse conditions. The amount of change brought about in yield, water requirement, and composition appears to be in direct relation to the duration and severity of infection. Crown rust affected significantly the yield and water requirement of both susceptible and resistant varieties when it appeared any time before the dough stage of development of the host. The longer the duration of infection the greater was the depreciation in yield, increase in water requirement, and change in chemical composition of the grain. The more severe the infection, as measured by percentage and type of infection, the greater also was the depreciation in yield, increase in water requirement, and change in chemical composition of the growing plant.

Crown rust reduced the yield of grain and roots of both susceptible and resistant varieties much more than that of the straw, which included the remaining parts of the plant. The effect on chemical composition of different parts of the plant was somewhat different. Green plants infected for one month and harvested at the boot stage of development showed the greatest change in composition due to the rust. The percentages of the sugars (sucrose, glucose, and levulose) were greatly decreased, along with a considerable decrease in dextrin, while the ash, nitrogen fractions, and acid-hydrolyzable substances were increased. The same trend was noted in the composition of the mature kernels from plants infected at different stages of development, *i.e.*, the nitrogen-free extract, which includes the sugars, dextrin, starch, etc., was slightly decreased, while the nitrogenous matter or crude protein was increased, although the differences were slight in comparison with those of the green plant. Crown rust apparently had no significant effect on the composition of the mature oat hulls.

That crown rust is located in the plant at the point where photosynthesis takes place gives it an excellent opportunity to avail itself of the newly synthesized foods before they are translocated to other parts of the plant. Certain of these, such as the sugars, were doubtless consumed by the fungus in respiration and, consequently, the total quantity of sugar and other carbohydrates was reduced, even though the mycelium and a small portion of the spores were included in the analysis. The rust organism must interfere with photosynthesis and other metabolic processes in many ways, such as mechanical interference brought about by rupturing the epidermis, ob-

structing the flow of liquids and passage of gases, reducing photosynthesis because of necrosis and chlorosis and by shading, etc., thereby limiting the amount of carbohydrates synthesized in the plant. This interference with photosynthesis, particularly for resistant varieties, probably is more injurious to the plant than the loss of the carbohydrates consumed by the fungus. The fact that the percentage of ash, insoluble solids, soluble nitrogen, and acid-hydrolyzable substances, such as cellulose, show relative increases is doubtless a result of smaller utilization of these substances by the fungus rather than to any actual increase due to the presence of the rust organism. Oats grown under field conditions and harvested for green foliage or for hay, in addition to having a considerable reduction in yield, also may be reduced greatly in feeding value because of crown-rust infection; while oats harvested for grain may show the effect of the rust mainly by a great reduction in yield, with little change in the feeding value of the grain.

Caldwell *et al.* (3) found that the percentage of crude protein in the grain of susceptible varieties of wheat was reduced significantly by heavy leaf rust infection under field conditions. Sucrose of the grain also was decreased, while the starch content varied inversely with the protein content. The results obtained in the present investigation, conducted under greenhouse conditions, are in agreement with theirs, except for the effect on the protein content of the grain. The percentage of crude protein in the kernels of susceptible and resistant oat varieties was slightly increased by heavy crown-rust infection, while the acid-hydrolyzable substances, which included the sugars, starch, and other carbohydrates, were slightly decreased. The authors cited obtained a higher percentage of nitrogen and a lower percentage of sucrose and reducing sugars also in the culms and leaves of rusted plants, which is in agreement with the results reported herein.

The data obtained in this investigation and another reported previously (7) emphasize the value of crown-rust resistant varieties, such as Bond and Victoria. Bond was resistant to all except 2 of the physiologic forms of crown rust collected in the years 1931–33, whereas Victoria was resistant to all of 37 forms collected in the period 1927–33. In addition, Bond is resistant to certain forms of loose and covered smut and Victoria apparently is resistant to the forms of loose and covered smut prevalent in the central and southern oat areas of the United States.

## SUMMARY

Under greenhouse conditions crown-rust infection, initiated in the anthesis stage on Markton (susceptible) and in the seedling, boot, and anthesis stages on Victoria (resistant), had no appreciable effect on the amounts of moisture, ash, fat, and fiber contained in the kernels. There was a slight increase in the crude protein as a result of infection, the amount of increase

apparently depending more upon the duration than upon the type of infection. Nitrogen-free extract was slightly decreased also in relation to duration rather than to type of infection. The composition of the hulls removed from these kernels was not appreciably affected by infection in any of the stages.

The green weight of infected plants of Markton and Iogold (both susceptible) and of Victoria and Bond (resistant and nearly immune, respectively) was 69.3, 63.6, 22.2, and 14.7 per cent lower, respectively, than that of rust-free plants.

Concentrations of insoluble solids, ash, nitrogen, and acid-hydrolyzable substances in the green plant, were all increased as a result of rust infection, the amount of increase being in relation to the susceptibility of each variety. Ammonia, amide, and nitrate and nitrite nitrogen showed the greatest percentage of increase. The increase in these non-colloidal nitrogen fractions for the susceptible Markton variety were 257.1, 327.3, and 322.0 per cent, respectively, while the greatest increase for the colloidal or protein nitrogen was 25.8 per cent. Corresponding maximum increases for insoluble solids and acid-hydrolyzable substances were 29.6 and 29.7 per cent, respectively.

The proportions of total soluble solids, sugars, and dextrin in the green plant were all decreased as a result of infection. The sugars showed the greatest decreases, sucrose, glucose, and levulose being decreased 83.6, 78.7, and 97.4 per cent, respectively, in the infected Markton plants, while total soluble solids and dextrin showed a corresponding decrease of 19.3 and 23.3 per cent, respectively.

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# REACTION OF CERTAIN VARIETIES AND SPECIES OF THE GENUS HORDEUM TO LEAF RUST OF WHEAT, PUCCINIA TRITICINA[1]

C. O. JOHNSTON[2]

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## INTRODUCTION

Varieties of barley have been used by many uredinologists to eliminate mixtures of leaf rust of wheat, *Puccinia triticina* Eriks. from cultures of stem rust, *P. graminis tritici* (Pers.) Eriks. and Henn. Complaints have occasionally been heard that leaf rust mixtures seemed to persist in some cultures despite the use of that method of purification. Field collections of leaf rust on barley, accompanied by the statement that the collector believed the rust to be leaf rust of wheat, occasionally also have been received by the writer. Most of these have proved to be the leaf rust of barley, *P. anomala* Rostr. One collection, however, was received from G. L. Peltier of the Nebraska Agricultural Experiment Station in 1927 that proved to be *P. triticina*. It gave a normal infection on seedlings of wheat and when transferred to the differential wheat varieties produced reactions typical for physiologic form 9. When transferred from wheat to seedlings of a local strain of White Hull-less barley, the infection produced was characterized by many small uredia surrounded by yellow necrotic areas. Thus there seemed some evidence that leaf rust of wheat could be propagated on certain varieties of barley.

An examination of the literature reveals that as early as 1911 Freeman and Johnson (3) stated that leaf rust of wheat attacked rye and barley to some extent, although Carleton (1) in earlier experiments had obtained negative results with barley. More recently, Stakman (9) stated that Schaal, a student in his laboratory, discovered that certain varieties of barley were moderately susceptible to *Puccinia triticina*. Waterhouse (10) reported in 1929 that *Hordeum murinum* and *H. maritimum* were susceptible to 2 physiologic forms of *Puccinia triticina* in Australia, while *H. jubatum* was immune; no mention was made of the reaction of varieties of cultivated barley. Hassebrauk (6) observed that form 14 of *P. triticina* caused

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pustule formation on *H. bulbosum, H. maritimum, H. spontaneum,* and several varieties of cultivated barley, while *H. jubatum, H. murinum,* and *H. secalinum* were highly resistant or immune. In the United States, Mains (7) reported negative results or only a trace of infection when *H. deficiens, H. distichon, H. gussoneanum, H. intermedium, H. jubatum, H. murinum, H. nodosum, H. pusillum,* and *H. vulgare* were inoculated with *P. triticina.* Cotter and Levine (2), working with *P. graminis secalis,* discovered that a large number of barley varieties exhibited varying reactions when inoculated with several physiologic forms of that fungus.

In view of the diversity of published reports, it seems desirable to record here the results of some experiments conducted at Manhattan, Kansas, on the reaction of cultivated varieties and wild species of barley to the leaf rust of wheat during the period 1928 to 1934, inclusive. These experiments were undertaken to gain some knowledge of the distribution of botanical types in the genus Hordeum capable of supporting a reproductive infection of *Puccinia triticina.*

## MATERIALS AND METHODS

Seed of the cultivated varieties was obtained from H. V. Harlan of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. Separate lots of seed were used for the experiments of 1928 and those of 1933. Seed of the wild species was furnished by W. M. Bever, Moscow, Idaho, G. A. Wiebe, Davis, Calif., and B. B. Bayles, Division of Cereal Crops and Diseases. That supplied by the latter was grown at the Arlington Experiment Farm, Rosslyn, Virginia.

The experiments were conducted in the greenhouse during the late fall and winter months at temperatures fluctuating between 65° and 85° F. An effort was made to keep night temperatures as near 70° F. and day temperatures as near 75° F. as possible. Six to ten barley seedlings were grown in 2-inch flower pots and inoculated about 8–10 days after emergence. In the experiments of 1928 the primary leaves of all seedlings were hand-inoculated, but in all later experiments seedlings in the two-leaf to four-leaf stage were first atomized with tap water and then inoculated by dusting with spores of the desired physiologic form. Inoculated plants were placed in moist chambers for 24 hours after which they were placed in full light on the greenhouse bench until ready for rust readings 10–14 days later. Inoculations with each physiologic form were repeated two or more times to make sure the proper reactions were being observed and recorded.

In making the rust readings, the infection produced by each physiologic form was recorded in terms of infection types as described by Mains and Jackson (8) in their studies on physiologic specialization of *Puccinia triticina* on wheat. The readings were based on the type of infection developed on the primary leaf although infection also sometimes was present on sec-

ondary leaves. Primary leaves often bore more and larger uredia than secondary leaves, although the latter also occasionally exhibited pustules as high as the third or fourth leaf. In some cases uredia developed on the primary leaf while only flecking was shown by secondary leaves.

## EXPERIMENTAL RESULTS

### Reaction of Cultivated Barley Varieties to *Puccinia triticina*

In 1928 seedlings of 51 varieties of cultivated barley, representing 18 botanical groups were inoculated with *Puccinia triticina,* physiologic form 9, as described above. The experiment was repeated twice during that season and the data recorded in table 1 are averages of the three readings. Perhaps the most important fact shown by this table is that none of the barley varieties was so susceptible to that form of leaf rust of wheat as were the wheat varieties Malakof and Regal, *i.e.,* none of the barleys was completely susceptible. On the other hand, several varieties of barley were more susceptible than the wheat variety Democrat, and several were as nearly susceptible as Hussar. It is perhaps inaccurate to speak of this type of reaction as susceptibility, for it actually is a moderate type of resistance in which there is a copious production of uredia that are small, usually are surrounded by yellow necrotic areas, and are accompanied by considerable flecking. Nevertheless, a number of barley varieties were hosts sufficiently favorable to permit reproduction by the fungus (Fig. 1).

The data in table 1 show that pustule formation is not limited to varieties in any one botanical group but occurs also on certain varieties of several groups. Ten of the 18 botanical groups were represented by only one variety and 2 others by only 2 varieties each. This is entirely too few to give anything like a true picture of the reaction of the group as a whole. Three groups, however, were represented by enough varieties to give a fair indication of their general reaction. These were *Hordeum vulgare pallidum, H. vulgare coeleste,* and *H. intermedium cornutum.* In the first of these Pannier C.I. 1330 was the only variety showing a moderate degree of pustule formation (type-2 infection). Of the 10 varieties in the *H. vulgare coeleste* group, there were 4 that exhibited an infection of type 2. The *H. intermedium cornutum* group exhibited the nearest approach to true susceptibility of any of those tested. Although all varieties of this group showed a moderate resistance (type-2 infection), there was a heavier production of uredia and these were somewhat larger and freer from chlorotic haloes than the pustules produced on most varieties of other groups. Three varieties of *H. deficiens* showed considerable pustule development in 1928 but failed to do so consistently in later experiments.

Fresh seed of a selected list of varieties was obtained and seedlings were tested with 6 physiologic forms of *Puccinia triticina* during the winter of

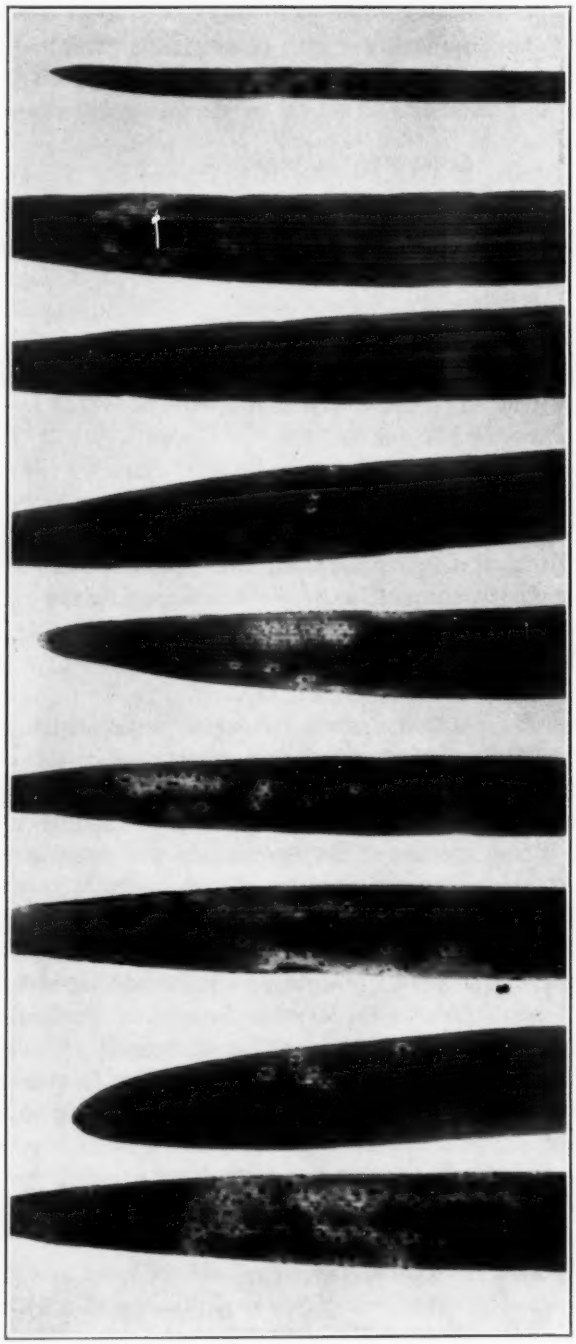

FIG. 1. Infections produced by *Puccinia triticina*, physiologic form 9, on the primary seedling leaves of eight varieties of barley and one variety of winter wheat in the greenhouse. The barley varieties from left to right are Cornutum C. I. 2366, Black Hull-less C. I. 666, Jet C. I. 967, Barley wheat C. I. 1384, Abyssinia C. I. 362, Hanna C. I. 203, Beldi Dwarf C. I. 190, and Oderbrucker C. I. 940. Winter wheat on the extreme right is Regal C. I. 7364.

TABLE 1.—*Reaction of barley and wheat varieties to physiologic form 9 of the leaf rust of wheat (Puccinia triticina) in the greenhouse. 1928*

| Variety[a] | C. I. No.[b] | Rust infection type | Variety | C. I. No. | Rust infection type |
|---|---|---|---|---|---|
| Hordeum vulgare pallidum | | | Hordeum vulgare trifurcatum | | |
| Beldi Dwarf | 190 | 0 | Skinless | 1032 | 0 |
| Club Mariout | 261 | 0 | Nepal | 1376 | 0–1 |
| Club Mariout | 2334 | 0 | Nepal | 2322 | 0 |
| Coast | 626 | 0 | Hordeum intermedium nudihaxtoni | | |
| Manchuria | 245 | 0 | Unnamed | 2352 | 1 |
| Mignon | 999 | 0–1 | Hordeum intermedium cornutum | | |
| Oderbrucker | 940 | 0–1 | Barley wheat | 1384 | 2 |
| Odessa | 927 | 0 | Cornutum | 2215 | 2 |
| Pannier | 1330 | 1–2 | " | 2323 | 2 |
| Trebi | 936 | 0 | " | 2427 | 2 |
| Hordeum vulgare nigrum | | | " | 1317 | 2 |
| Gatami | 575 | 0 | " | 2366 | 2 |
| Hordeum vulgare nigrum leiorrhynchum | | | Hordeum distichon palmella White Smyrna | 658 | 0–1 |
| Lion | 923 | 0–1 | Hordeum distichon palmella nutans | | |
| Hordeum vulgare horsfordianum | | | Chevalier | 1419 | 0 |
| Horsford | 507 | 0 | Hanna | 203 | 0–1 |
| Hordeum vulgare coeleste | | | Hordeum distichon palmella erectum | | |
| Ak Arpa | 747 | 0–1 | Svanhals | 187 | 0 |
| Dehra | 1085 | 0–1 | Hordeum distichon nigricans | | |
| Hansee Hull-less | 703 | 2 | Black Smyrna | 191 | 0–1 |
| Hull-less | 745 | 2 | Hordeum distichon nudum | | |
| India Hull-less | 698 | 0–1 | Evans | 621 | 0 |
| Kama-ore | 694 | 2 | Hordeum distichon nudumianthinum | | |
| Kharsila | 2318 | 2 | Irisaka | 1083 | 1–2 |
| Semet | 1314 | 0 | Hordeum deficiens nigrinudum | | |
| Yane-hadaka | 693 | 0 | Jet | 967 | 1–2 |
| Yane-hadaka | 2319 | 0–1 | Hordeum deficiens steudelii | | |
| Hordeum vulgare coeleste himalayense | | | Abyssinia | 362 | 2– |
| Ederle | 1015 | 0 | Steudelii | 2226 | 1–2 |
| Gorak | 1086 | 1–2 | Triticum vulgare | | |
| Himalaya | 620 | 0 | Malakof | 4898 | 4 |
| Hordeum vulgare coeleste violaceum | | | Hussar | 4843 | 2 |
| Black Hull-less | 1106 | 2 | Mediterranean | 3332 | 0 |
| " " | 596 | 0–1 | Regal | 7364 | 4 |
| " " | 666 | 1–2 | | | |
| " " | 1027 | 0–1 | | | |
| Spain | 1370 | 0–1 | | | |

[a] Varieties classified according to Harlan (5).

[b] C. I. denotes accession number of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

1933. The results obtained are recorded in table 2. These data show clearly that the three varieties of *Hordeum intermedium cornutum* that were tested approached susceptibility in several instances. This group as a whole and the variety Black Hull-less C.I. 666 of *H. vulgare coeleste*

TABLE 2.—*Reaction of a selected group of barley and wheat varieties to six physiologic forms of the leaf rust of wheat (Puccinia triticina) in the greenhouse. 1933*

| Variety | C.I. No. | Type of infection[a] produced by form number | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 5 | 9 | 15 | 33 |
| Hordeum vulgare pallidum | | | | | | | |
| Beldi Dwarf | 190 | 0 | 0 | 0 | 0 | 0 | 0 |
| Oderbrucker | 940 | 0 | 0 | 0–1 | 0 | 0 | 0–1 |
| Hordeum vulgare coeleste violaceum | | | | | | | |
| Black Hull-less | 666 | 2– | 2 | 2 | 2+ | 2– | 2+ |
| Hordeum intermedium cornutum | | | | | | | |
| Barley wheat | 1384 | 2+ | 2+ | 2++ | 2+ | 2+ | 2+ |
| Cornutum | 2287 | 2 | 2 | 2+ | 2+ | 2+ | 2+ |
| Cornutum | 2366 | 2– | 2– | 2 | 2++ | 3– | 2++ |
| Hordeum distichon palmella nutans | | | | | | | |
| Hanna | 203 | 0 | 0–1 | 0 | 0 | 0 | 0 |
| Hordeum deficiens nigrinudum | | | | | | | |
| Jet | 967 | 0 | 0 | 0 | 2– | 0–1 | 0–1 |
| Hordeum deficiens steudelii | | | | | | | |
| Abyssinia | 362 | 0 | 0 | 0–1 | 0–1 | 0–1 | 0–1 |
| Steudelii | 2226 | 0–1 | 0 | 0–1 | 0–1 | 0 | 0–1 |
| Triticum vulgare | | | | | | | |
| Malakof | 4898 | 0 | 0–1 | 4 | 4 | 0 | 0 |
| Hussar | 4843 | 1 | 4 | 1 | 2– | 2= | 2= |
| Mediterranean | 3332 | 0 | 4 | 4 | 0 | 4 | 4 |

[a] Plus and minus signs are used to indicate tendencies toward a somewhat higher or lower infection type than the type indicated by the numeral. The sign of equality denotes a double minus.

*violaceum* were only moderately resistant to all six of the forms, while all other varieties were very highly resistant to all forms with the single exception that Jet C.I. 967 was only moderately resistant to form 9. Although there was some variation in the reaction of each of the three varieties of *H. intermedium cornutum* and the variety Black Hull-less to the various physiologic forms, the differences were not great enough to be of differential value or of much significance. The greatest difference in reaction was exhibited by Cornutum C.I. 2366 on which form 15 produced a 3-minus-type infection, while forms 1 and 2 produced only a 2-minus-type. The 3-minus infection was the nearest approach to true susceptibility observed in these studies. The variety Barley wheat C.I. 1384 exhibited the least resistance to all of the 6 forms, while Beldi Dwarf C.I. 190 was the most resistant to all. Despite the fact that Black Hull-less C.I. 666, Barley wheat C.I. 1384, Cornutum C.I. 2287, and Cornutum C.I. 2366 all bore infections in which

uredia were copiously produced when inoculated with the 6 physiologic forms, none of them could be considered susceptible to *P. triticina* in the same sense that the wheat varieties Malakof and Mediterranean were susceptible to some of the forms. They were, however, just as nearly susceptible as was the wheat variety Hussar to forms 9, 15, and 33, and much more nearly susceptible to form 1 than any of the 3 wheat varieties.

## Reactions of Certain Wild Species of Hordeum to Physiologic Forms of *Puccinia triticina*

Since some groups of the cultivated barleys seemed to be more favorable hosts for *Puccinia triticina* than others, it was decided to ascertain the response of certain species of wild barleys to several physiologic forms of that fungus. In the genus Triticum high susceptibility to leaf rust is most frequently encountered in the species having 42 somatic chromosomes, while those with lower chromosome numbers are more resistant. It seemed possible that the same thing might hold true for the genus Hordeum. Griffee (4) has shown that all of the cultivated barley groups, *Hordeum spontaneum*,

TABLE 3.—*Reaction of seedlings of several species of wild barley from various sources to the leaf rust of wheat (Puccinia triticina) in the greenhouse. Manhattan, Kans., 1934*

| Species | C.I. No. | Source of seed[a] | Infection type produced by form number | | |
|---|---|---|---|---|---|
| | | | 5 | 9 | 15 |
| H. murinum | 6069 | California | 0 | 0 | 0–1 |
| " " | | Idaho | 2– | 2 | 2= |
| " " | 4102 | Virginia | 0 | 0 | 0 |
| " " | 4278 | " | 2= | 2= | 1 |
| " spontaneum | 2294 | " | 2= | 2= | 2= |
| " gussoneanum | 6067 | California | 2 | 2 | 2– |
| " " | 6066 | " | 1= | 1 | 1 |
| " nodosum | 6072 | " | 0–1 | 0 | 0 |
| " " | | Idaho | 0 | 0 | 0 |
| " maritimum | | " | 2 | 1+ | 1+ |
| " bulbosum | 6071 | California | 0–1 | 2= | 2– |
| " jubatum | 6070 | " | 0 | 0 | 0 |
| " " | | Idaho | 0 | 0 | 0 |
| " pusillum | 6078 | California | 0 | 0 | 0 |
| " " | | Kansas | 0 | 0 | 0 |
| " " pubens | 6068 | California | 0 | 0 | 0 |

[a] Seed was supplied by G. A. Wiebe, Davis, Calif., W. M. Bever, Moscow, Idaho, and B. B. Bayles, Div. of Cereal Crops and Diseases, United States Department of Agriculture, Washington, D. C. (seed from Arlington Experiment Farm, Rosslyn, Va.). The Kansas collection was made locally by the author.

*H. maritimum* and *H. Caput-Medusae* have only 14 somatic chromosomes, while *H. jubatum* and *H. murinum* have 28, and *H. nodosum* has 42.

Seed of species of wild barley was obtained from 3 different sources, and seedlings were grown in the greenhouse and inoculated with physiologic forms 5, 9, and 15 during the winter of 1934. The results obtained are given in table 3. The best development of uredia was obtained on *Hordeum gussoneanum* and two strains of *H. murinum*. On these the very numerous uredia were small and accompanied by chlorosis. It is interesting to note that both highly resistant and slightly susceptible strains appeared in *H. murinum* and *H. gussoneanum*. The California strain of *H. murinum* was definitely more resistant to all 3 rust forms than the Idaho strain, although the plants were morphologically indistinguishable. On the other hand the two strains of *H. gussoneanum* differed somewhat in certain plant characters as well as in their reactions to the three physiologic forms of leaf rust.

The reactions of the two strains of *Hordeum murinum* from Arlington Farm are of particular interest because differences between them had already been noted in plant characters. The strain C. I. 4278 was labeled "small seed" and the seedlings were definitely shorter in stature than those of the strain C. I. 4102. The small-seed type was a definitely more favorable host for all 3 physiologic forms than the large-seed strain. The latter exhibited nothing but flecking in all cases, while uredia of all forms developed on the small-seed type.

A few of the species seemed to have a slight differential reaction to the 3 physiologic forms of rust. For example, *Hordeum maritimum* was somewhat less resistant to form 5 than to forms 9 and 15, and *H. bulbosum* was more resistant to form 5 than to forms 9 and 15. In general, however, the differences were not distinct enough to be of much significance or of any differential value.

From the data obtained it seems clear that the nearest approach to susceptibility was not encountered in the species with the largest number of chromosomes (*Hordeum nodosum*). As a matter of fact that species was strongly resistant to all 3 physiologic forms. In the jubatum-chromosome group, which has 28 somatic chromosomes, *H. jubatum* was very highly resistant to all 3 forms, while *H. murinum* contained some strains that were very highly resistant and others that were only moderately resistant. Two wild species of the vulgare-chromosome group, having 14 chromosomes, were tested. One of these, *H. spontaneum*, was uniformly resistant to all forms but permitted a considerable development of uredia. The other, *H. maritimum*, was only moderately resistant to form 5 but rather highly resistant to forms 9 and 15. It is clear, therefore, that high resistance is found in all of the chromosome groups and that the weakest resistance appeared in the

groups having 14 and 28 chromosomes. The number of species tested is, however, too small to justify any general conclusions on the relation of chromosome number to resistance to *Puccinia triticina*. There is some evidence that a relationship exists and that the nearest approach to susceptibility can be expected in species with the lower chromosome numbers. Despite this fact, however, it must be kept in mind that very highly resistant and nearly susceptible varieties occur among the botanical groups of cultivated barleys all of which have 14 chromosomes.

## DISCUSSION

The results of these studies would seem to indicate that no varieties of cultivated barley and none of the wild species of Hordeum are completely susceptible to the leaf rust of wheat. They also demonstrate that certain varieties and species approach susceptibility to an extent where there is a rather copious production of small uredia. Whether the propagation of leaf rust of wheat on barley would ever be of any economic significance is unknown to the writer. The data show that certain of the cultivated varieties and wild species of barley are capable of supporting enough uredia to aid in the dissemination and propagation of wheat leaf rust. Should some of the winter varieties prove to be as nearly susceptible as the spring varieties tested in these studies, they might be considered an aid in the overwintering of *Puccinia triticina*. The only winter variety tested in these experiments was a local strain of Tennessee Winter that proved to be rather strongly resistant to all 6 physiologic forms. Considerable flecking occurred but only a few small uredia developed and the variety could not be considered nearly so favorable a host as some of the spring varieties. Spring seedings of the more susceptible varieties might aid in the propagation of leaf rust of wheat in some parts of the United States, but the writer has not observed that condition in the Great Plains and adjacent areas.

The data on the reaction of barley varieties to *Puccinia triticina* show that considerable care must be exercised in choosing a variety for use in removing mixtures of that rust from cultures of *P. graminis tritici*. The leaf rust of wheat sporulates on some varieties of barley almost as freely as does the stem rust of wheat.

Considering the difference in the reaction to leaf rust of certain varieties of cultivated barley and of different strains of some of the wild species, it is not surprising that the results obtained by Hassebrauk (6), Waterhouse (10), and others differed. The results obtained in the studies reported herein indicate that with but few exceptions wider differences could be expected from the inoculation of different varieties and strains of barley with a single form, than from the inoculation of a single variety or strain with several physiologic forms.

Among the cultivated barleys the production of uredia was noted in several botanical groups, but it seems to be most marked in the group *Hordeum intermedium cornutum*. Although the number of varieties tested in some of the groups was too small to permit a general statement concerning group reactions, it seems safe to say that pustule formation is not confined to any one group and probably occurs in all groups.

In general there was very good agreement between the results obtained with the cultivated barleys in 1928 and in 1933. One exception worthy of note is the reaction of the two varieties of *Hordeum deficiens steudelii* to form 9 in the two experiments. In 1928 these varieties exhibited a considerable production of uredia, while in 1933 they exhibited mostly flecking with the occurrence of an occasional minute uredium. Such a difference might have been due to differences in greenhouse conditions, but that seems doubtful. It seems more likely that it was due to a slight difference in the rust culture or the seed. Although form 9 was employed in both cases, the culture used in 1933 was of different origin than that used in 1928. It was necessary to obtain a new supply of seed of the varieties used in 1933 and there is a possibility that there was a slight physiologic difference, although the varieties were botanically the same.

## SUMMARY

The response of seedlings of certain cultivated varieties and wild species of barley to several physiologic forms of the leaf rust of wheat, *Puccinia triticina*, was studied in the greenhouse.

No cultivated variety or wild species of barley proved to be fully susceptible. However, several economic varieties and some strains of wild species permitted a copious production of small uredia and, therefore, might be considered a factor in the propagation of leaf rust of wheat.

Some barley varieties cannot safely be used to purify cultures of stem rust of wheat because of their tendency to bear reproductive infections of *Puccinia triticina*.

Pustule formation to some extent appeared in nearly all of the botanical groups of cultivated barley but seemed to be most abundant on varieties of *Hordeum intermedium cornutum*.

Greater differences in response to wheat leaf rust were observed when several varieties were inoculated with a single form than when one variety was inoculated with several different physiologic forms.

Among the species of wild barley *Hordeum gussoneanum* C. I. 6067 presented the nearest approach to susceptibility. *H. spontaneum*, *H. maritimum*, *H. bulbosum*, and a strain of *H. murinum* from Idaho exhibited a limited pustule development when inoculated with 3 physiologic forms. *H. nodosum*, *H. jubatum*, *H. pusillum*, and *H. pusillum pubens* were very highly

resistant to all forms, while *H. murinum* and *H. gussoneanum* had highly resistant and moderately resistant strains.

The greatest number of varieties and species exhibiting pustule formation occurred in the 14- and 28-somatic-chromosome groups. *Hordeum nodosum*, with 42 chromosomes, was highly resistant. This is the reverse of the condition in the genus Triticum where the greatest number of resistant varieties falls in species having the lower chromosome number, and most of the highly susceptible varieties occur in the vulgare group having 42 somatic chromosomes.

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# CONTROL OF SOIL FUNGI BY SOIL FUMIGATION WITH CHLOROPICRIN

G. H. GODFREY[1]

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The control of soil-borne organisms injurious to plants constitutes one of the problems in the field of plant pathology not yet satisfactorily met. This paper constitutes the complete report (8) on preliminary studies that have indicated that, by means of an efficient soil fumigant that is both fungicidal and insecticidal in its action, some of the objections inherent in standard methods of soil disinfection may be overcome.

## LITERATURE DISCUSSION

For the control of greenhouse-bed and seed-bed organisms injurious to plants, most standard practices involve either a liquid-fungicide, nematicide, or insecticide drench, alone, or combined, or a heat treatment of some sort. The literature is extensive and no effort is made herein to cover it in full. For the control of damping-off organisms in seed beds, various metallic salts of high fungicidal efficiency have been advocated, including those of copper, zinc, and mercury, the latter in both inorganic and organic compounds. Sulphuric acid and acetic acid also have been used for this purpose. All of these require drenching of the soil, and effectiveness in killing the soil fungi extends only so far as the chemical in sufficient concentration penetrates. Guba (14) has demonstrated the efficiency of carbon bisulphide emulsion + formaldehyde in the control of nematodes and Fusarium in greenhouses. The cost of this combined treatment is about $160 per 10,000 square feet or approximately $700 per acre. Unless the soil is exceptionally well drained, several days or even weeks will be required for elimination of surplus water and chemicals before a crop can be planted. This objection, of course, inheres in the use of any soil-drench treatment.

Bulletins by Newhall (17) and Newhall and Chupp (18) on soil-sterilization methods include the various methods of applying heat. Steam treatments have given excellent results in nematode control and increased yields in tomatoes and other crops, but they involve high expense in the matter

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of installation of equipment, or in labor costs in case installations are not permanent. In Wisconsin costs up to or exceeding $1000 per acre are common for heat treatments of tobacco and nursery seed beds. Recently developed German machines (1, 23) would appear to give satisfactory steam treatments to soils, but the soil must be handled and moved for the operation. Hot-water treatment carries with it the objection of water-logging the soil, if poorly drained, besides being rather expensive in coal and labor costs.

The use of fumigants for the disinfection of soils has received considerable attention. Hydrocyanic acid gas has been advocated for years for nematode control (5, 16, 25), and Baudys (2) claims for it a certain fungicidal effect. Carbon bisulphide, likewise, has long been used against nematodes (4) and to a certain extent against soil fungi (7), though as a rule it appears to be lacking in effectiveness in the latter respect (14). Chloropicrin has recently (10, and references) been indicated as a decidedly promising chemical for the control of nematodes. In field experiments with pineapples, Godfrey (10) has demonstrated significantly better results with it than with either hydrocyanic acid gas or carbon bisulphide. Roark (20) lists several papers in which the bactericidal and fungicidal effectiveness of chloropicrin is indicated. Cooke (6) attributes a stimulation of sugar-cane growth to the control of a root infecting Pythium. Schepetilnikova (22) reports on the use of this chemical in combating flax sickness. Oserkowsky (19) gives the results of laboratory experiments with various volatile chemicals, including chloropicrin, bromopicrin, and others, in which several of the gases under full vapor pressure at room temperature kill the dry sclerotia of *Sclerotium rolfsii*. Chloropicrin was not so effective as bromopicrin.

The advantage of the so-called "partial sterilization" of soil, with a resulting apparent stimulation of plant growth, following treatment with chloropicrin, has been pointed out by Russell (21). Godfrey and his collaborators (12) also have reported a marked stimulation of growth, particularly in tomatoes. They also have emphasized the advantage to be gained from adequate confinement of a fumigant, which is reflected in much enhanced fumigation efficiency. Any fumigant of high vapor pressure rapidly volatilizes and escapes from the soil unless held in by a highly impervious cover. Not only does adequate confinement make for better penetration of the gas into soil clods and the like, as well as to greater depths, but it increases the period of exposure of organisms to the action of the gas; and time is definitely a factor in killing. Godfrey (9) has developed a glue-coated paper that effectively confines chloropicrin gas.

## EXPERIMENTS

The experiments here recorded relate solely to soil fumigation with chloropicrin with a view to determining its fungicidal efficiency. A technique is followed similar to that used in nematode-control experiments (12).

*Soil.* A prepared greenhouse soil consisting of well-screened, loose sandy loam, about 1/6 sand, was used. At the time of the test it contained about 8 per cent moisture on the air-dry-weight basis; well below field capacity, but well above the wilting point. This amounts to about 10 per cent water, by volume. The air-dry soil contained almost exactly 50 per cent air space, as measured by water displacement. The slightly moist soil as used, then, contained air in the proportion of about 40 per cent of the total soil volume. The soil was rendered completely sterile, as shown by culture tests, by autoclaving twice on successive days for 2½ hours at 15 pounds' pressure. This was for the purpose of simplifying reisolation attempts, after fumigation.

*Containers.* The vessels used for the experiments were 4-gallon glazed stone jars. These were filled to ½ inch from the tops with soil.

*Fungus Cultures. Fusarium sp.*: isolated from gladiolus; supplied by A. W. Dimock of the Division of Plant Pathology of the University of California.

*Phytophthora cactorum*: isolated from snapdragons; a culture supplied by J. T. Barrett, obtained originally from M. R. Harris of the California State Department of Agriculture. The transfer used was contaminated with bacteria, but the Phytophthora was vigorous, and showed abundant cottony growth at the surface.

*Rhizoctonia solani*: from sugar beet; culture from J. T. Barrett.

*Sclerotium rolfsii*: from sugar beet. Free sclerotia were used, just as taken from culture tubes of potato-dextrose agar.

*Verticillium albo-atrum*: from strawberry; obtained from Harold E. Thomas.

*Dematophora sp.*: isolated from apple roots, transferred to sterilized apple twigs; culture obtained from Harold E. Thomas.

*Armillaria mellea*: isolated from prune roots, transferred to sterilized apple twigs upon which it grew until the fungus was evident in a stroma-like growth at the distal end from point of inoculation.

*The fumigant,* chloropicrin, was applied at the rate of 1¼ cc. or 2.07 g. per jar; equivalent to approximately 400 pounds per acre-foot of soil. Of the total volume of the jar, 4 gallons, or 15,140 cc., only 40 per cent, or 6056 cc. is air space. According to the Chemical Warfare School, Edgewood Arsenal, New Jersey (24), the maximum possible concentration of gas obtainable at 20° C. and standard pressure is 0.164 grams per liter of air. The air in the jar, then, would be saturated with gas with $6.056 \times 0.164$, or 0.993 grams of chloropicrin. Actually, therefore, an excess of 2.07/0.993, or 2.1 to 1 was used, disregarding adsorption by soil particles and solution in the soil water. This figure was checked very closely by a rough calculation based upon the proposition that a gram molecule of a gas will occupy 22.4 liters under standard

conditions of temperature and pressure, the gas in this case, of course, being mixed with air at 760 mm., its own vapor pressure being 18.3 mm. at 20° C. The adsorption factor was not calculated. The solubility of chloropicrin in water is 0.14 per cent. With the great amount of water surface exposed in soils, then, a considerable proportion of the available chloropicrin might conceivably be taken up by the soil water. This probably contributes to, rather than detracts from, the effectiveness of the chemical in gas form, for, as shown by Bertrand and Rosenblatt (3) only 5 parts per million in water (which is 1/300 of saturation) stops yeast fermentation entirely. Considering adsorption by the soil and solubility in soil water, then, the soil air in this experiment may or may not have been saturated with chloropicrin gas.

*Technique of Manipulation of the Cultures.* Small glass cylinders about ½ cm. long and 1 cm. in diameter were filled with loose soil and covered tightly with cheese-cloth tied in place with a string with identifying label attached. These were autoclaved, within Petri dishes. Using aseptic methods, cultures of the various fungi were inserted into the centers of the cells, one to each cell, and a drop of sterile water added to insure the presence of sufficient moisture for fungus growth. The cells, with labels on long strings, were thrust into the jars of soil to about 6 inches depth at points around the periphery. The technique with the root-rotting fungi, Dematophora and Armillaria, differed from the foregoing in that the inoculated twigs were thrust directly into the soil, without the glass cells. The cultures were allowed to incubate in the soil for 24 hours, before fumigation.

*Technique of Fumigation.* Using a previously marked pipette for exact measurement, chloropicrin was taken from a dropper bottle and inserted in a hole near the center of the jar to a depth of 3 inches, and covered quickly with soil. Immediately the top of the jar was sealed by gluing over it a circular piece of glue-coated paper cut to size, and sealing it perfectly tightly at the edge with a strip of highly impervious Scotch masking tape, 2 inches wide. There was no opportunity for escape of the gas through any opening at the edges. The only escape that could occur was directly through the paper, and this, as shown by previous tests (9), was possible at only a very slow rate. The test was conducted in duplicate. Duplicate jars with identical arrangement of cultures but without the fumigant, were prepared as controls. Exposure to the fumigant was for a period of 48 hours.

When the covers were removed from fumigated jars, the odor of gas was strong at the surface and in samples of soil lifted from different depths in the jars, showing that penetration of the gas through the soil was efficient.

*Culture Attempts.* The cultures were lifted by their strings and placed in sterile Petri dishes with loose covers and left there for 24 hours to allow escape of excess chloropicrin possibly still in the cells. It was noted at this time that in the case of the culture of *Rhizoctonia solani*, the period of 24

hours of incubation before fumigation had been sufficient to permit of growth of fungus hyphae, not only throughout the soil in the cell, but into surrounding soil as well, so that surrounding soil was actually lifted with the culture. Transfers were made from bits of the original inocula and surrounding soil to poured plates of potato-dextrose agar.

*Results.* All fumigated cultures were completely negative for growth up to 10 days after plating, with one exception. The bacterium-contaminated culture of *Phytophthora cactorum* showed growth of the bacteria only. It would appear that this was a resistant, probably spore-bearing soil bacterium. This verifies to that extent the conclusion of Russell (21) and others as to the "partial sterilization" effect of chloropicrin on soils. All control cultures grew normally. Microscopic examination of the profuse hyphal growth of the rhizoctonia culture, that had occurred before fumigation, disclosed a collapsed condition with the protoplasm not evident. Similar hyphae from the control culture were normal. Figure 1 shows Petri-dish cultures from this experiment.

## ADDITIONAL TESTS AND OBSERVATIONS

*Phytophthora cactorum in Snapdragons.* In a large commercial greenhouse near San Leandro, Alameda County, Calif., the phytophthora disease on snapdragons, described by Harris (15), had increased to an alarming degree in the season of 1934 over what had been present the year before. With one variety in particular the mortality was about 25 per cent. In another greenhouse the loss was nearly 100 per cent. By way of a simple experiment to test chloropicrin treatment as a control measure, the writer took soil from beneath wilted plants and placed it in stone jars. One of these was fumigated, as in the foregoing experiment; one was left as a control. The two soils were placed in flats and planted to snapdragon plants pricked from a flat of seedlings. Two weeks after planting, 14 plants of the 56 in the flat of nontreated soil had collapsed and showed typical phytophthora injury at the soil line. At the same time the treated flat showed a 100 per cent vigorous stand. Later, however, one of the plants in this flat failed; still later, 2 or 3 more. Meanwhile, the percentage of failure in the nontreated soil had increased to 50 or more. The infection in the treated flat may have been due to contamination from the other flat, as the two were side by side. Striking efficiency of the chloropicrin fumigation was demonstrated, however.

*Verticillium Wilt in Tomatoes.* In another experiment, soil from a greenhouse showing abundant verticillium wilt of tomatoes was similarly treated. Tomato plants transplanted from a flat of greenhouse soil in which no Verticillium had ever been observed, in the plant pathology greenhouse at the University of California, grew vigorously at first in treated and nontreated soils alike. When the plants were about 15 inches high symptoms of wilting

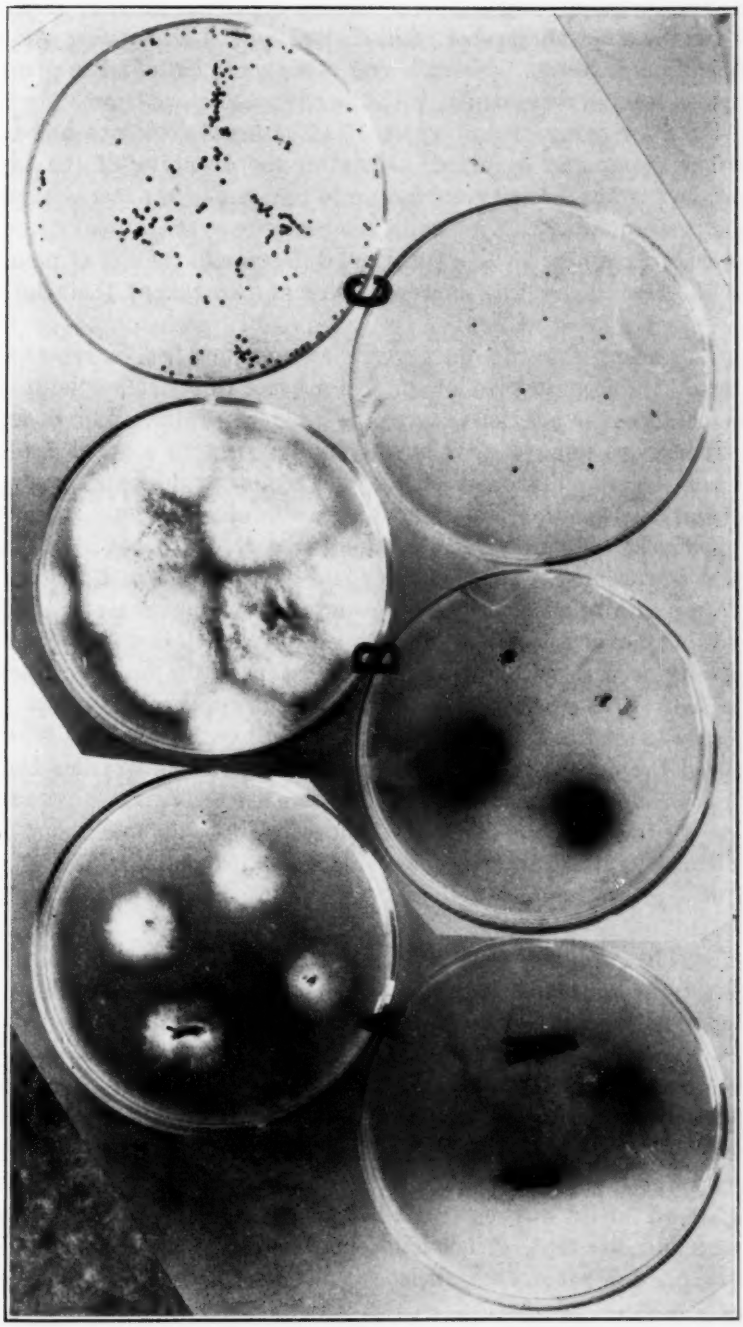

FIG. 1. Results of a fumigation test with chloropicrin. The plates on the top, from nonfumigated fungi; those on the bottom from fumigated ones. A. *Dematophora* sp. on apple twigs. B. *Fusarium* sp. from gladiolus. The dark stain is from the pigment of leaves. C. *Sclerotium rolfsii*. Note complete freedom from growth of sclerotia from the fumigated culture and the abundant reproduction of sclerotia in the nonfumigated culture. All other cultures tested showed results that were just as definitely positive as those illustrated.

occurred in one of the nontreated pots. Shoots cut from the side of this plant and from the other nontreated plant showed typical brown-ring symptoms of verticillium infection. No such symptoms could be found in plants from the treated soil. Subsequently, with the advent of warmer weather in late spring and after new root development had occurred from procumbent stalks, recovery apparently occurred, and, after the dropping of the first wilted leaves, no further wilting occurred up to the time of the last observations. As is well known, with a rise in temperature verticillium-infected plants frequently recover in part. A distinct difference in the size of plants in favor of the treated lot was, however, evident throughout their later growth.

A final experiment, this time in a greenhouse ground-bed, is reported, mainly to record the technique followed. In a house in which verticillium wilt had been observed to a serious degree the previous winter, a plot of soil $6 \times 8$ ft. in dimensions was set apart by surrounding it with a 6-inch board frame sunk into the ground about 5 inches. The soil, after the usual manure fertilization and deep spading, was treated by means of a Vermorel Injector with chloropicrin at the rate of 400 pounds per acre, at 1 foot intervals. Immediately a glue-coated paper cover was laid over the plot and sealed to the board frame with an efficient paste, leaving no opening whatever. An additional sealing measure consisted in wetting down the soil in a furrow just outside the frame, with a view to preventing the escape of the gas too quickly through the soil. This was kept wet by occasional applications of water during exposure to the fumigant. The cover was left on for 6 days. When it was removed the odor of the gas was still strong, demonstrating high efficiency in gas confinement. Tomato plants were set according to regular practice, a day or two after the cover was removed. Up to the latter part of June, about 3 months after planting, the plants in treated and adjoining nontreated soil had grown equally well and were approximately 6 feet high. Again the warm summer conditions were unfavorable to the development of Verticillium as it had occurred the previous winter. However, several plants in the untreated portion of the house showed definite wilting of some leaves and a few plants were completely collapsed. No plants in the treated soil showed any symptoms of verticillium infection.

*Disinfection of Greenhouse Potting Soils.* In an attempt to determine the practicability of the disinfection of greenhouse potting soils with chloropicrin, the writer had made some special boxes, as containers of such soils, in which, directly, the fumigation treatment was to be given. The boxes were made air tight and the lid was constructed to fit tightly to permit of sealing the cracks with adhesive tape. When chloropicrin was applied at the standard rate, $2\frac{1}{2}$ cc. per cubic foot, high efficiency was demonstrated in the killing of injurious soil fungi, nematodes, garden centipedes, collembolids, wire-

worms, sowbugs, and the like, and even, to some extent, of weed seeds. The killing of nut-grass, *Cyperus rotundus*, corms by chloropicrin fumigation has already been demonstrated (12).

*Chloropicrin for Sterilizing Glassware.* The writer has found that ordinary laboratory glassware, such as Petri dishes, can be very efficiently sterilized by stacking them dry in a container that can be tightly closed, such as a coffee can, and putting a few drops of chloropicrin into one of the dishes, then sealing the container tightly with adhesive tape. The quantity of the fumigant used needs theoretically to be only 0.1 cc. to each liter of air space, though doubling or tripling this quantity may be desirable in practical application, in order to insure continued saturation of the atmosphere. A few hours to a day probably is sufficient period of exposure. The dishes must be permitted to stand in an open chamber for 2 or 3 hours for aeration before using for culture work. The equipment for this may prove to be a very satisfactory supplement to the apparatus of a traveling plant pathologist for its obvious advantages of convenience and ease of manipulation.

## DISCUSSION

Several factors are to be considered in connection with the use of chloropicrin as a soil fumigant for whatever purpose.

(1) It possesses the property, rare among chemicals, of being simultaneously and without special modification, a fungicide, a nematicide, and an insecticide.

(2) It requires no wetting of the soil. The quantity of water consumed in a drench treatment is no small factor. Guba states that 1000 gallons of liquid are necessary for 1000 square feet of surface.

(3) There is no injurious residue left in the soil. Planting can be done within a day or two after treatment.

(4) Plantings of tomatoes by the writer have never shown any of the toxic effects frequently observed from heat-sterilized soils. Instead, there is often an apparent stimulation of plant growth, such as that reported following "partial sterilization" of soils.

(5) The cost, at the current price of about $1.00 per pound of chloropicrin, is slightly less than 1 cent per square foot of surface for the fumigant. The cost of a gas-tight paper should not exceed about 1/5 cent per square foot. The total cost of treating 1000 square feet, then, would be:

| | |
|---|---|
| Chloropicrin | $10.00 |
| Paper | 2.00 |
| Labor, about | .50 |
| Total | $12.50, or $125 for 10,000 square feet. |

The paper can be used repeatedly, if handled carefully. The cost of chloropicrin may be greatly reduced if an extensive application for it in this direction is developed. A recent quotation of $0.65 per pound in large quantities has been made by one manufacturer. A lower rate of application may prove equally effective. Three hundred pounds per acre gave as good results as higher rates for nematode control (10).

*Precautions.* For indoor application, such as in greenhouses, it is advisable to use a gas mask. For out-of-door work, with ordinary precautions, this is not necessary. Likewise, for greenhouse work, this material should not be used in a portion of the house occupied in any part by growing plants. It has been found (11) that relatively low concentrations of the gas in the air are injurious to many plants.

The fact of great reduction or complete elimination of beneficial legume bacteria by chloropicrin fumigation of the soil (12, p. 1340) should be considered, if a legume is to be planted. With sweet peas, for example, while good control of Rhizoctonia is to be expected, the supply of Rhizobium should be renewed by seed or soil inoculation if good plant growth be expected.

The rate of diffusion of chloropicrin through a continuous layer of water is very low. The soil must not be too wet, as the gas will not diffuse efficiently into soil that lacks open air-spaces between the soil particles. A primary consideration in the use of chloropicrin as a soil fumigant, therefore, is that the soil must be in a state of good tilth and on the dry side rather than the wet.

The need for the best possible confinement of the gas in the soil can not be too greatly emphasized. The vapor-pressure gradient between the point of application and the open air is high, 18.3 to 0, and there will be constant diffusion in the direction of escape from the soil unless there is an adequate impermeable cover holding it in. The gradient within the soil is the same at the start and it gradually becomes less with adsorption of the gas by soil particles, solution in the soil water, complete vaporization at the source, and the inevitable escape of gas, until equilibrium is reached. With the release of the gas by removal of the cover the gas is quickly evacuated from the soil. Complete freedom from any break in the confining cover, obviously, is an important consideration. The value of a supplementary barrier of wet soil around a treated plot, to prevent the escape of gas through the soil at the edge, is also evident. Unless the presence of gas in the soil be readily detectable by odor at the end of a period of exposure of perhaps 48 hours, it may be taken for granted that the escape of gas has been too rapid and best efficiency can not be expected.

*The Fungicidal Value of Other Fumigants.* Whether or not other fumigants have fungicidal value equal to that of chloropicrin remains to be determined by specific trial. Hydrocyanic acid gas, carbon bisulphide, tetra-

chlorethane, and ethylene dichloride are to be considered in this connection. Gersdorff's (13) bibliography on the latter chemical contains 4 citations that mention the killing of certain pathogenic bacteria, and yeasts. Its general fungicidal value may, therefore, be worthy of special study.

SUMMARY

Laboratory experiments designed to determine the practical fungicidal value of chloropicrin used as a soil fumigant were conducted with 4-gallon glazed, stone jars of soil. Fungi tested were *Fusarium* sp. from gladiolus, *Phytophthora cactorum* from snapdragons, *Rhizoctonia solani* from sugar beet, *Sclerotium rolfsii* from sugar beet, *Verticillium albo-atrum* from strawberry, *Dematophora* sp. from apple roots, and *Armillaria mellea* from prune roots. The cultures were inserted adjacent to the wall of the jar to a depth of 6 inches, then chloropicrin was applied at the rate of 1¼ cc. per jar (equivalent to 400 pounds per acre foot) into the center of the jar. Gas-impervious, glue-coated paper was used to seal the jars. Exposure was for a period of 48 hours. Cultural attempts on plates of nutrient agar were completely negative for growth for all fumigated cultures. Controls were all positive. Subsequent small-scale tests with greenhouse soils naturally infested with plant-disease fungi gave good indications, as did also a greenhouse ground-bed test with tomato Verticillium. The practicability of disinfecting greenhouse potting soils, in special boxes, was demonstrated. Incidentally, the feasibility of sterilizing laboratory glassware by chloropicrin fumigation was likewise shown.

UNIVERSITY OF CALIFORNIA,
BERKELEY, CALIF.

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# A BACILLUS ISOLATED FROM DISEASED PLANTS OF AUCUBA JAPONICA (THUNB.)[1]

GEORGE TRAPP, M.A., B.Sc., PH.D.

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## INTRODUCTION

During the investigation of a die-back disease of the Japan laurel a bacillus was constantly isolated from stem, leaf, and root lesions of affected plants. Since the organism possesses characters that clearly distinguish it from other forms previously described in bacteriological science and since these differences are of specific rank, the life history of the bacillus has been worked out in some detail.

## ISOLATIONS

The organism was obtained as a halo of pure growth, (Fig. 1, A), surrounding sections of diseased leaves, stems, and roots implanted on the sterile surface of plated nutrient agar or gelatin, after thorough surface sterilization of the organs from which the sections were taken. Similar surface-sterile sections cut from corresponding organs of healthy Aucuba plants and planted as controls on like nutrient media gave rise to no bacterial growth, thus ruling out the possibility of an epiphytic or commensal origin for the organism.

## INOCULATION EXPERIMENTS

Although the presence of the organism internally in the more recently necrosed tissues of Aucuba shoots, affected by a blight of the die-back type and accompanied by comparable root lesions, might be taken as an indication of etiological relationship, yet exhaustive attempts to reproduce the symptoms of disease by artificial inoculation failed to establish the pathogenicity of the organism in spite of the adoption of a varied technique and the employment of a diversity of experimental material and conditions involving the inoculation of upwards of 150 plants and cuttings. In all inoculations young and vigorous subcultures from recent isolations were used, the pure cultures of the organism being obtained from different organs and from different plants supplied from a number of distinct localities where the incidence of the disease was observed to be particularly severe.

Under the circumstances it seems advisable to regard the organism as a saprophyte especially adapted to form the vanguard of secondary invaders that dispose of the débris provided by the progress of the primary lethal

[1] Excerpt from thesis approved for the degree of Doctor of Philosophy by the University of Glasgow.

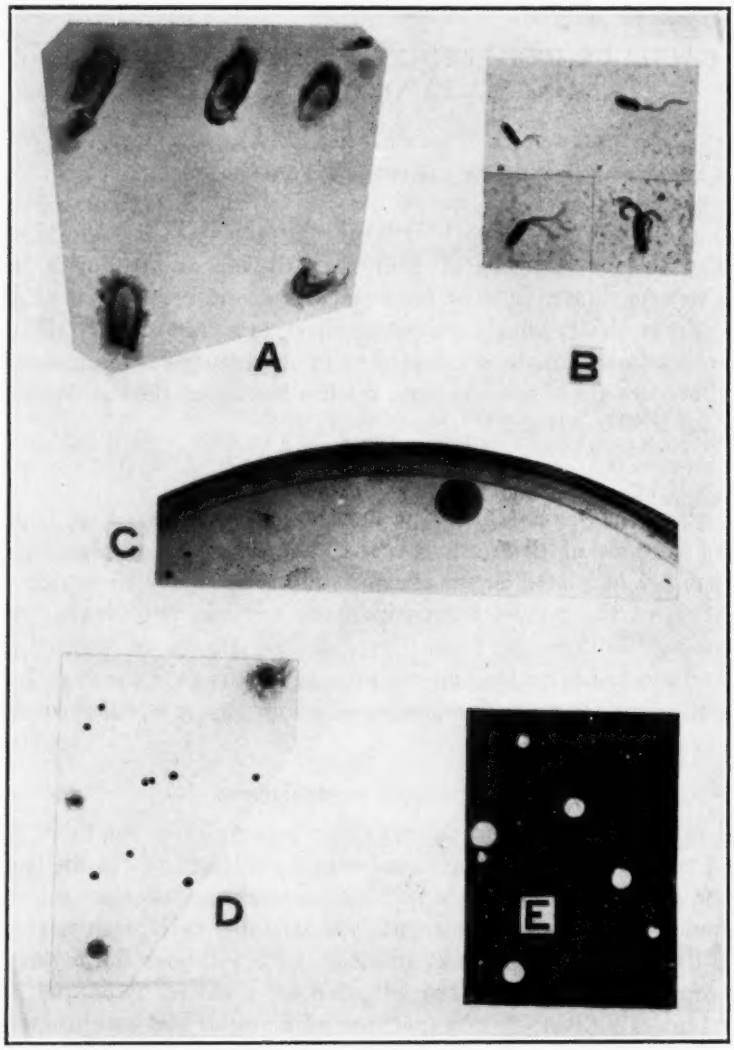


FIG. 1. *Pseudomonas aucubicola*, sp. nov. A. Poured plate isolation of the organism by halos of pure growth from stem sections of diseased aucuba twigs implanted on agar. ×0.7. B. Film preparation of the bacillus from a 2-day-old agar streak culture. The rods possess from 1 to 4 polar flagella. Stained by Kirkpatrick's method. ×1,420. C. Surface and buried colonies of the organism on beef-infusion agar. Photographed by oblique transmitted light from a 3-day-old culture to show homogeneous structure and entire margin of surface colony. ×1.6. D. Surface and buried colonies of the organism on gelatin. The surface colonies show large areas of surrounding liquefaction; the buried colonies are small and their position marked by the occurrence of small pits of liquefaction at the surface of the medium. Age of culture and photography as in C. ×1.3. E. Surface and buried colonies of the organism on gelatin after prolonged subculturing on agar. Note the raised growth of the surface colonies and the absence of liquefaction. Photographed by reflected light from a 3-day-old culture. ×0.9.

agent whose nature has so far evaded elucidation. This tentative conclusion appears the more correct in view of the failure to detect any sign of the organism in appropriately stained microtome sections taken from the junction of healthy and diseased tissues in material that, prior to fixation, showed an actively advancing margin of necrosis.

### LIFE HISTORY OF THE AUCUBA BACILLUS

*Morphology.* The Aucuba organism is a short, usually slightly curved, rod with rounded ends, occurring singly or in pairs frequently arranged to form an obtuse angle. Smears from 3 days' old cultures on beef-infusion agar stained with carbol fuchsin give rods varying in length from 0.90 μ to 1.70 μ and in diameter from 0.45 μ to 0.60 μ and with an average measurement of 0.60 μ by 1.20 μ. In broth culture the corresponding measurements are 1.70 μ to 3.40 μ and 0.60 μ to 0.85 μ and an average size of 0.65 μ by 2.60 μ. No capsules are produced by the organism on agar or in broth when films are stained by Hiss's method. Examined in wet preparations, the organism exhibits a darting or "vibrioid" type of motility and, stained by Kirkpatrick's method[2] is shown to possess 1 to 4 lophotrichate flagella (Fig. 1, B). No endospores have been observed.

*Staining Reactions.* The organism is definitely gram-negative and is not acid-fast. It stains well with carbol fuchsin but not so intensely with the aniline dyes. Stained with Loeffler's flagellum stain and with the metachromatic Carbol Thionin refractive granules were sometimes observed at one or both poles of the bacillary body: these granules did not stain as glycogen with iodine.

### CULTURAL CHARACTERS[3]

*Beef Infusion-agar Slants.* In tubes held at 20° C. the growth along the strokes is moderate to strong, whitish gray in color, slightly convex, translucent at the margin, becoming opaque towards the centre; surface smooth, glistening; margin smooth, with a tendency to flattened undulations; of viscid consistency. A few tiny pits in the surface were frequently observed with the aid of a lens. In old cultures (several months) spiked branched crystals were formed expanding from the surface growth into the medium.

*Beef Infusion-agar Plates.* On poured plates surface colonies can be detected with the naked eye on the second day and by the sixth day have reached a diameter of 4 mm.; the maximum diameter attained after 10 days, was 11 mm. The surface colonies are at first circular and homogeneous by transmitted light (Fig. 1, C); they are slightly raised, whitish grey in color

[2] Muir, R., and J. Ritchie. Manual of bacteriology. Ed. 9. 866 pp. Oxford University Press. 1932.

[3] Unless otherwise stated the organism was cultivated at 20° C.

with moist glistening surface and smooth, entire margin; later, (8 days onwards), they appear flatter and by reflected light a number of faint slightly raised concentric rings and a few slightly depressed radiate markings are observable; the margin remains entire but is now possessed of major undulations. The buried colonies are small (less than 1 mm. after 6 days) lenticular and brown with an opaque centre and a more translucent regular margin, which becomes granular as the colony ages without increase in size.

*Beef-extract (Lemco) Agar Slants and Plates.* On this medium the form of the growths obtained was similar to that on beef infusion agar.

*Beef-extract (Lemco) Broth.* The medium is clouded, most densely towards the surface, after 24 hours. By the second day rim and pellicle formation commenced. The lacy pellicle, though connected with the rim, was easily detached by the slightest agitation and even in undisturbed tubes older cultures showed abundant sediment derived from the dejection of the surface skin.

*Potato Slopes.* The growth on potato is decidedly brown from the commencement and is fairly abundant though not covering the entire surface of the potato. Growth tends to be somewhat heaped along the strokes; the consistency is viscid; the surface is glistening, smooth and the margin entire but thrown into arcs. Abundant precipitate occurs at the bottom of the water which is otherwise clear. The substance of the potato is discolored throughout to a brown which is less intense than the pigmented growth.

## PHYSIOLOGICAL PROPERTIES

*Chromogenesis.* When grown on beef infusion media the organism produces a light green, water-soluble, extracellular pigment diffusing into the medium of agar slants, which it impregnates completely by the third day. With subsequent growth or aging of the culture, pigmentation is not intensified. No color is formed from media prepared with beef extract (Lemco).

*Liquefaction of Gelatin.* When the organism is isolated from the host onto gelatin plates and tube cultures inoculated from single colonies, typically liquefying colonies are apparent in the dilution plates by the second day. On the sixth day, the surface colonies, now 4–5 mm. in diameter, are surrounded by a circular area of liquefaction (Fig. 1, D). The colony at this stage, when photographed by oblique transmitted light, consists of a dense white center with zooglœal threads spreading into the liquefied halo, but to the actual margin of liquefaction bacterial growth does not appear to have penetrated. The buried colonies are minute (about 0.5 mm. after 6 days at 20° C.) and brownish opaque with a somewhat diffuse discontinuous margin, the position of the colonies being indicated by depressions at the surface of the medium (Fig. 1, D).

*Gelatin Stabs.* In stab cultures inoculated immediately from gelatin isolations, liquefaction is rapid; at first napiform, it later becomes saccate and proceeds to completion in 14 days and sometimes even less.

*Loss of Power of Liquefaction.* On account of the diagnostic and taxonomic importance attached to this property, the following account of alteration in the organism's behavior in this respect, consequent on its prolonged cultivation on agar, may be considered relevant. It was observed that poured gelatin plates inoculated with a culture that had been held on agar through successive transfers gave raised, white, opaque, non-liquefying colonies (Fig. 1, E) of an anomalous (for gelatin) type. In order to determine whether a non-liquefying variant had been segregated or whether the change could be explained on physiological grounds alone, the following experiment was undertaken. A culture of the organism, which for convenience may be designated A, obtained from the liquefied material of a gelatin stab was plated out on gelatin and a typical colony (all the colonies were of the liquefying type) was used to inoculate an agar slope on January 8. After repeated subculturing on agar, poured plates were made from a recent transfer (of March 21) on March 24. After 2 days numerous colonies of the raised circular opaque type were visible on dilutions I and II, but the medium showed no signs of incipient liquefaction. The same procedure was repeated with a parallel culture B obtained from a raised non-liquefying colony on gelatin dilution culture derived from an isolation that had been grown previously on agar for many generations. The inoculum from colony B was first subcultured on agar slants on January 8. After successive transfers it finally was plated out on gelatin on March 24 from a subculture of March 21. By March 26 typical raised colonies had appeared that in growth-form and absence of liquefaction were indistinguishable from those obtained simultaneously and under the same environmental conditions from the culture described above under A. Several days later, however, it was observed that both A and B colonies were sinking slightly into depressions caused by the slow liquefaction of the medium. A final observation on April 12 showed that the gelatin on all A and B plates had been completely liquefied. It is concluded, therefore, that the organism's power to cause liquefaction is, if not permanently destroyed, at least lost temporarily and modified to such an extent by growth on agar as to render diagnosis on this basis uncertain unless the cultural history of an isolation can be specified exactly. Morphologically and physiologically, therefore, except in respect of gelatin, the rapidly liquefying and slowly liquefying forms of the organism are identical.

Gelatin tubes stabbed from cultures isolated on agar and subsequently grown on that medium give entirely different characters from those described previously under gelatin stabs, where the organism was isolated on and inoculated from gelatin. Liquefaction is slow to commence (about the 4th day)

and forms at first just a shallow depression where the surface was punctured and into which the growth sinks. The liquefaction then becomes stratiform and slowly descends into the tube at the rate of 4 mm. after 6 days in a typical case, 13 mm. after 18 days, 25 mm. after 38 days, and after 9 weeks the gelatin was less than half liquefied. On the surface of the solid gelatin below the liquefied zone there was a copious flocculent deposit in a conical heap; rim and reticulate pellicles were formed, the latter being easily precipitable; the liquefied portion of the gelatin was clear and, with beef infusion medium, showed green pigmentation which, however, did not diffuse into the solid gelatin in a manner similar to growth on agar.

*Fermentation of Sugars and Alcohols.* The following carbohydrates were prepared in concentrations of 1 per cent with peptone water as basic medium: glucose, saccharose, lactose, raffinose, mannite and dulcite. The fermentable solutions were set up with Durham's fermentation tubes and Andrade's indicator. Inoculated from a young agar slant culture and observed every 24 hours up to 4 days and then at intervals of 6 days up till the second month, the tubes at no time showed any indication of the production of acid or gas from the test substances, although in every case the solutions showed evidence of growth with rim and pellicle formation and a moderate amount of sediment. There was no turbidity at the closed ends of the fermentation tubes.

*Litmus Milk.* In litmus-milk the litmus is reduced without previous change of reaction. The milk at first forms a finely flocculent coagulum and is later completely peptonized. In one example no change was observed till the sixth day, when a rim and sediment had formed and the litmus showed reduction by forming zones with a clear layer commencing at the top. After 22 days a thick creamy layer was present to a depth of 11 mm. from the surface; this was followed by a reduced clear zone of 13 mm., then 13 mm. of incompletely reduced litmus succeeded by a uniform zone extending to the bottom of the tube, which contained an abundant precipitate. After 40 days the litmus was completely reduced and showed no later tendency for the color to return: the milk was completely digested and the whey clear.

*Hydrolysis of Starch.* Although the organism had no markedly disintegrative effect on potato cylinders after 30 days' growth, the starch of a beef-extract-broth culture containing 1 per cent soluble starch was completely hydrolyzed after 19 days when tested with iodine-potassium iodide solution and compared with noninoculated control tube.

*Reduction of Nitrates.* Nitrates are not reduced.

*Production of Ammonia.* A trace of ammonia is produced from beef-extract (Lemco) broth (containing peptone) as detected by inserting into the tubes strips of filter paper previously dipped in Nessler's solution.

*Production of Hydrogen Sulphide.* Hydrogen sulphide is not produced. There was no blackening following stab inoculations of lead acetate agar.

*Voges and Proskauer Reaction.* Acetyl-methyl-carbinol is not produced.

*Production of Indole.* Indole is not produced. Peptone water cultures, 2 days and 24 days old, were tested with Ehrlich's rosindol reagent and a saturated watery solution of potassium persulphate: the sodium nitrite-sulphuric acid method also was applied: both sets of tests gave negative results.

*Toleration of Sodium Chloride.* Grown in beef-extract broth containing sodium chloride in concentrations of 1, 2 and 3 per cent and examined after 3 days the organism shows a definite, uniform clouding with rim and pellicle formation in the 1 per cent NaCl medium. In 2 per cent NaCl broth turbidity was uniform only for the uppermost 7 mm.; below, the strands of growth spread down into the medium and finally disappeared, leaving the lower half of the tube unclouded: no rim or pellicle was formed in this case although a few floccules floated at the surface. With 3 per cent NaCl broth there was no turbidity. The same 3 cultures, examined after 20 days, showed growth and the occurrence of sediment in all the tubes, but a comparison of the amount of the deposit formed by the organism in the 3 cases showed that it was inversely proportional to the concentration of NaCl in the respective cultures. Therefore, while growth is not entirely inhibited by a concentration of 3 per cent, the organism is regarded as being sensitive to the presence of sodium chloride.

*Relation to Free Oxygen.* The organism is an obligatory aërobe. Agar slant tubes, inoculated and placed in a Buchner's tube, the contained atmosphere of which is deprived of its oxygen by a mixture of pyrogallic acid and caustic soda, show no growth, however long anaërobic conditions are maintained. (These anaërobic cultures were retained and examined for signs of growth up to 11 months). No growth occurs along the track of the needle in deep agar and gelatin stab inoculations sealed over, immediately after inoculation, with 10 c.c. of the melted medium. Ordinary stab cultures in deep agar tubes show no growth along the track of the inoculating wire except for the first 2 cm. below the surface, where there was a slight, granular, discontinuous nonspreading growth. The surface of the medium around the stab, however, was completely covered with a thick, smooth, glistening whitish grey growth with adherent rim. The first examination of one such stab culture was made 6 days after inoculation and later after 18 days, but there was no observed increase in the amount or change in the appearance of the scanty subsurface growth. As noted above, there was no clouding in the closed ends of fermentation tubes (Kühn's and Durham's).

*Relation to Reaction of Medium.* The occurrence of growth on both decidedly acid and decidedly alkaline agar media indicates for the organism a fairly wide range of accommodation to the concentration of hydrogen ions: *e.g.*, abundant growth is obtained on media neutral to phenolphthalein (pH about 8.4). The organism cannot be regarded, therefore, as very sensitive to considerable alterations in the pH of the medium.

*Temperature Relations.* The optimum temperature for growth lies between 20° and 25° C. Growth occurs at 37° C. but it is very thin, flat and serous, and at this temperature the organism produces no green pigment on beef-infusion media. The maximum temperature is about 40° C. and the thermal death point in the region of 50° C.

*Longevity on Artificial Media.* Subcultures from liquefied gelatin show that the organism is fairly long-lived (4 to 6 months) on this medium. Cultures on agar slopes, however, appear to be much shorter lived, as a specific example will indicate. A subculture of August 23 when used to inoculate an agar slant on September 24 gave no growth at room temperature, but a loopful of the original culture suspended in beef broth gave a slight turbidity. On placing loopfuls of the broth subculture on an agar slant, typical growth was obtained. The foregoing experience demonstrates the fact that in this instance very few of the bacteria remained viable after a month's cultivation on beef agar.

*Technical Description.* Rods, slightly curved, with rounded ends, 0.45 to 0.60 by 0.90 to 1.70 μ, occurring singly or in pairs. Motile by 1 to 4 polar flagella. Nonsporiferous: nonencapsulated: nonacid-fast: gram-negative.

Gelatin stab: liquefaction saccate when inoculated from isolations in gelatin: cultivation on agar greatly diminishes this power of liquefaction but it returns slowly to a stratiform type. Liquefied medium greenish.

Agar colonies: circular, whitish grey, slightly raised, smooth, glistening, amorphous and of about 5 mm. diameter by the 6th day.

Agar slant: smooth, glistening, fairly abundant, whitish grey growth of viscid consistency. Beef-infusion media diffused with green water-soluble pigment: no chromogenesis occurs on beef-extract ("Lemco") media.

Broth: turbid, denser at first towards the surface: rim and pellicle formation (after 2nd day): sedimentation occurs later.

Litmus milk: peptonized slowly to completion, with zonal reduction of litmus, in 4 to 5 weeks.

Potato: growth moderate, somewhat raised, brown, viscid, with smooth, glistening surface and entire margin: medium of a less intense brown than pigmented growth.

Indole is not formed.

Nitrates are not reduced.

No acid or gas from glucose, saccharose, lactose, raffinose, mannite and dulcite (in peptone-water medium).

Starch hydrolysis moderate.

Ammonia production doubtful.

Acetyl-methyl-carbinol is not produced.

Hydrogen sulphide is not produced.

Sodium chloride, in concentrations of 1, 2 and 3 per cent in broth caused

retardation of growth proportional to the concentration of salt present, *i.e.*, sensitivity to the presence of sodium chloride is indicated.

Aërobic, obligatory.

Optimum temperature 20° to 25° C. Thermal death point about 50° C.

Habitat: isolated from the recently decayed tissues of roots, stems and leaves of the Japan laurel.

## CONCLUSION

A careful comparison of the characters of nearly related species of Pseudomonas, as recorded in the Fourth Edition of Bergey's Manual,[4] with those of the organism described in the present work showed that the latter possessed specific differentia. **Pseudomonas aucubicola,** sp. nov., is therefore offered as the name of the organism. It may also be pointed out that no previous mention has been made of Aucuba as a bacterial host plant (*vide* Elliott's list of Bacterial Plant Pathogens [and saprophytes]).[5]

## SUMMARY

A motile bacillus, specifically characterized as **Pseudomonas aucubicola,** sp. nov., was consistently isolated in pure culture from the more recently necrosed portions of stem, leaf and root lesions on plants of *Aucuba japonica* Thunb., affected by blight symptomatically distinguished by local die-back of twigs and root terminals and in some cases by acronecrosis of individual apical leaves.

The morphological, cultural, and physiological aspects of the organism's life history are described in detail.

In view of the lack of positive result from carefully planned and comprehensive inoculation experiments and the failure to detect its presence in suitably stained histological preparations of tissue in active necrosis, the organism is provisionally regarded as a saprophyte of special habitat.

## ACKNOWLEDGMENTS

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BOTANY DEPARTMENT,
UNIVERSITY OF GLASGOW,
GLASGOW, SCOTLAND.

[4] Bergey, D. H. Manual of determinative bacteriology. Ed. 4. London. 1934.

[5] Elliott, Charlotte. Manual of bacterial plant pathogens. London. 1930.

# STUDIES ON THE ORIGIN OF YELLOW-MOSAIC VIRUSES[1, 2]

JAMES H. JENSEN

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## INTRODUCTION

Evidence that has been accumulating during the past few years indicates clearly that closely related strains of tobacco-mosaic virus exist in nature. This is also true of cucumber-mosaic virus and latent-mosaic virus of potato. Whether these viruses have long existed as they are found today, or whether new strains are continually arising, is a question that has received some consideration in the literature but concerning which there is still much need for further study. This question is of importance, not only from a theoretical viewpoint regarding the nature of viruses, but also from a practical standpoint, since plants affected by attenuated or mild-disease-producing strains of certain viruses acquire immunity from more severe strains of the same viruses. The occurrence of yellow-mosaic viruses in irregular-shape bright yellow spots that appear occasionally on mottled leaves of tobacco plants infected with tobacco-mosaic virus seems to offer a means for studying further the question of origin of strains of tobacco-mosaic virus.

The evidence obtained previously (4) regarding the origin of yellow-mosaic viruses may be summarized briefly as follows: The viruses were isolated from plants infected with tobacco-mosaic virus that had been repeatedly transferred from single necrotic lesions on leaves of *Nicotiana glutinosa* L. plants. It was believed that repeated passage of tobacco-mosaic virus through single necrotic lesions would serve to free it from all traces of yellow-mosaic virus that might have been present originally. It was concluded from these experiments that yellow-mosaic viruses arise during multiplication of tobacco-mosaic virus in infected plants.

It is the purpose of this paper to report the results of further experiments designed to test the validity of this conclusion. These additional experiments were concerned with the questions: whether bright yellow spots would appear on tobacco plants inoculated with samples of tobacco-mosaic virus believed to be derived from single infectious units; whether the yellow-

[1] The experimental work reported here was carried out in the laboratories and greenhouses of The Rockefeller Institute for Medical Research at Princeton, New Jersey, and of the Department of Plant Physiology at the University of Wisconsin. The writer acknowledges with pleasure his indebtedness to Dr. B. M. Duggar and Dr. L. O. Kunkel for criticism and guidance throughout the course of this investigation.

[2] Published at the expense of The Rockefeller Institute for Medical Research, Princeton, N. J., out of the order determined by date of receipt of the manuscript. This practice in nowise delays the publication of manuscripts printed at the expense of The American Phytopathological Society or other agency.

mosaic viruses isolated from such bright yellow spots were strains of tobacco-mosaic virus; and whether these yellow-mosaic viruses would, in turn, give rise to other viruses.

## MATERIALS AND METHODS

The tobacco-mosaic virus used was derived from virus contained in a single necrotic lesion on a leaf of *Nicotiana langsdorffii* Schrank. The lesion resulted from a single pin-puncture inoculation of tobacco virus 1 (5), a sample of which was kindly supplied by James Johnson, of the University of Wisconsin.

The experimental attempts to obtain single infectious units of virus fall roughly into two types: (A) those dependent entirely on mechanical action, *i.e.*, high dilution and single pin-puncture inoculation; (B) those in which the virus remaining after some special treatment was inoculated into leaves of *Nicotiana langsdorffii* plants and subsequently transferred from the necrotic lesions produced. The lesions on *N. langsdorffii* are large (Fig. 1) and virus is relatively easily recovered from them. It is believed that single necrotic lesions result, in most cases, from inoculation with single infectious units of virus (Kunkel 6).

The tobacco plants used were *Nicotiana tabacum* L. var. Turkish. Unless otherwise specified, all plants were grown in fertilized soil in 4-inch or 6-inch unglazed clay pots or in shallow wooden flats. The greenhouse temperature during the course of experiments reported varied from about 68° F. to about 90° F., with an average temperature of about 72° F.

*The rubbing method* of inoculation (Holmes 3) was used in all gross transfers of virus. Other special methods of inoculation used have been described previously (4).

## YELLOW-MOSAIC VIRUSES IN PLANTS INOCULATED WITH VIRUS BELIEVED TO HAVE BEEN DERIVED FROM SINGLE INFECTIOUS UNITS

Attempts were made to obtain further information regarding the origin of yellow-mosaic viruses through the use of tobacco plants inoculated with tobacco-mosaic virus samples believed to be derived from single infectious units. A record was kept of the yellow spots that appeared on these plants and of the yellow-mosaic viruses isolated from the spots. The attempts to obtain single infectious units of virus and the results of inoculations with samples of virus derived from them are described in the following experiments.

*Isolation by Means of High Dilution.* Expressed juice from diseased tobacco plants was diluted 1: 10,000,000 with distilled water. A sterilized cheesecloth pad was then saturated with the diluted juice and rubbed over one leaf on each of 1247 healthy young Turkish tobacco plants. Within 14

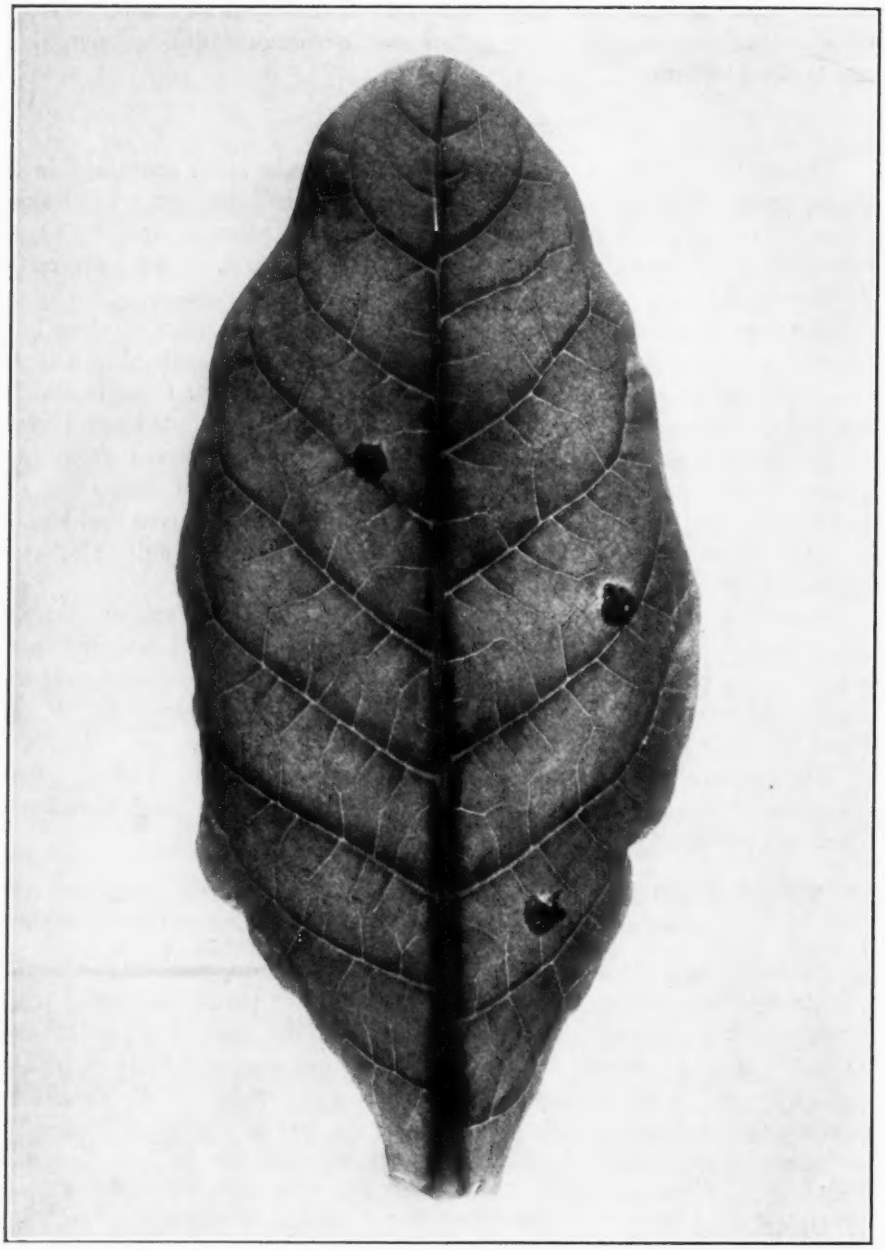

FIG. 1. Necrotic primary lesions on a leaf of *Nicotiana langsdorffii*. Isolated necrotic lesions are believed to be the result of inoculation with single infectious units.

days after inoculation, a total of 24 plants showed typical symptoms of tobacco mosaic infection. Eight days after inoculation, bright yellow spots appeared on some of the infected plants. Within 40 days after inoculation, all diseased plants showed one or more of these spots. Single pin-puncture isolations were made from bright yellow spots on 2 different plants, and 2 unlike yellow-mosaic viruses were recovered. They are of the "slow-moving" type; in tobacco they move from the point of inoculation so slowly that they rarely reach the tip leaves. They usually produce blotch-like yellow primary lesions only. Both viruses are difficult to transmit by simple rubbing methods when in undiluted juice, and even more difficult to transmit after dilution.

The development of one or more typical bright yellow spots on leaves of every infected plant and the demonstration that yellow-mosaic viruses were present in some of these spots show that such viruses occur in tobacco plants inoculated with juice containing tobacco-mosaic virus diluted 1:10,000,000.

*Isolation by Means of Single Pin-puncture Inoculation.* From a diseased leaf that showed no bright yellow spots, single pin-puncture transfers were made to small tip leaves of 100 healthy young tobacco plants. Within 7 days, 55 of these inoculated plants showed typical clearing-of-veins symptoms of tobacco-mosaic infection. Nine days after inoculation, the first bright yellow spot appeared. Within 44 days, one or more yellow spots had appeared on every diseased plant. Yellow-mosaic viruses were isolated from 4 bright yellow spots in this set of plants. The experiment furnished evidence that yellow-mosaic viruses occur in tobacco plants infected with tobacco-mosaic virus by single pin-puncture inoculation.

*Isolation by Means of Ultrafiltration.* Ten cc. of frozen juice of diseased tobacco plants were added to 35 cc. of 0.1 M $Na_2HPO_4$ solution and passed through a Seitz filter under pressure. Ten cc. of the filtrate were then passed through a collodion membrane, the average pore size of which, as determined by rate of water flow, was about 90 mμ. According to experiments reported by Thornberry (15), this pore size in collodion membranes permitted the passage of a very limited number of tobacco-mosaic particles. The collodion membrane filtrate was rubbed over 3 leaves of each of 16 *Nicotiana langsdorffii* plants. A total of 10 necrotic lesions appeared on the 48 leaves rubbed. Untreated, expressed juice from diseased plants rubbed over the leaves of 3 control plants produced over 1000 lesions per inoculated leaf, whereas untreated healthy juice rubbed over the same number of leaves produced no necrotic lesions. Virus from each of the 10 necrotic lesions when transferred to a healthy young tobacco plant produced typical symptoms of tobacco-mosaic infection. Within 40 days after inoculation, one or more bright yellow spots appeared on each plant. Yellow-mosaic virus was isolated from 3 of these spots.

This experiment demonstrated that yellow-mosaic viruses were present in tobacco plants inoculated with samples of tobacco-mosaic virus that had passed membranes with pore sizes approaching the limit that permits virus particles to pass.

*Isolation by Treatment with Chemicals.* Further attempts to obtain samples of tobacco-mosaic virus believed derived from single infectious units were made by testing partially inactivated samples of virus that had been treated with certain chemicals (chloramine-T, lead acetate, silver nitrate, and potassium permanganate) known to act deleteriously (14). In these experiments the chemicals were made up in solutions double the strength indicated in table 1. The treatments were carried out by mixing equal

TABLE 1.—*Number of necrotic lesions obtained on 9 leaves of Nicotiana langsdorffii rubbed with diseased juice treated with various chemicals*

| Substance added | Final concentration | No. lesions | Final concentration | No. lesions |
|---|---|---|---|---|
| Chloramine-T | 2%[a] | 420[b] | 4% | 13[c] |
| Lead acetate | 1% | 103 | 2% | 90[c] |
| Silver nitrate | 4% | 10[c] | 8% | 0 |
| Potassium permanganate | 1% | 455 | 2% | 70[c] |

[a] Obtained by mixing equal volumes of solution, double the concentration indicated, and clarified diseased juice.

[b] 7500 necrotic lesions were produced when an equal number of leaves were rubbed with untreated diseased juice diluted in 1 part water.

[c] Virus from 10 single necrotic lesions was transferred to 10 healthy young tobacco plants.

volumes of the solutions and expressed diseased tobacco juice, partially clarified by freezing and centrifugalization. After standing for a period of one hour, the deleterious materials were removed from the solutions by dialysis and the remaining treated juice rubbed over leaves of *Nicotiana langsdorffii* plants. In each instance, as shown by table 1, the number of necrotic lesions obtained by inoculation with treated juice was much smaller than that obtained by inoculation with nontreated juice. Virus from 10 single necrotic lesions obtained by inoculation with samples of juice treated with each of the 4 chemicals was transferred to 10 tobacco plants. Within 50 days after inoculation, one or more bright yellow spots had appeared in every tobacco plant inoculated with virus from a single necrotic lesion. Single pin-puncture inoculations demonstrated that virus of yellow mosaic was present in 11 of the bright yellow spots tested.

Expressed juice of diseased tobacco plants was adjusted to pH 1.0 and pH 11.5 by the addition of 2 N HCl and 2 N NaOH, respectively. Stanley (13) has described the rate of virus inactivation at these hydrogen-ion concentrations. Samples of treated juice, taken at various intervals after the beginning of the treatment, were neutralized and then rubbed over leaves of *Nicotiana langsdorffii* plants. Table 2 presents the number of necrotic

TABLE 2.—*Number of necrotic lesions obtained on 9 leaves of Nicotiana langsdorffii rubbed with neutralized samples of 1:3 diluted diseased tobacco juice which had been held for various lengths of time at one or the other of two hydrogen-ion levels*

| | | | | | |
|---|---|---|---|---|---|
| Diseased juice adjusted to pH 1.0 by addition of 2 N HCl | | | | | |
| No. hours treated | ¼ | 24 | 48 | 54 | |
| No. lesions obtained | 1410 | 885 | 230 | 120[a] | |
| Diseased juice adjusted to pH 11.5 by addition of 2 N NaOH | | | | | |
| No. minutes treated | 1 | 15 | 25 | 35 | 45 |
| No. lesions obtained | 870 | 241 | 176 | 38 | 26[a] |

[a] Virus from 10 single necrotic lesions was transferred to 10 healthy young tobacco plants.

lesions obtained in the various inoculations. Virus from 10 single necrotic lesions that resulted from infection with the samples of partially inactivated inoculum was transferred to young tobacco plants. In all instances bright yellow spots appeared on leaves of the infected tobacco plants within 50 days after inoculation. It was demonstrated by means of single pin-puncture inoculations that virus of yellow mosaic was present in at least 7 of the bright yellow spots.

Expressed juice of frozen diseased tobacco plants was diluted 1:3 with water, adjusted to pH 3, treated with commercial pepsin at the rate of 10 mg. per cc., and incubated at 35° C. Stanley (12) has reported the proteolytic action of this enzyme on tobacco-mosaic virus. At various intervals after the beginning of treatment, samples of treated virus were neutralized and inoculated into leaves of *Nicotiana langsdorffii*. Table 3 shows the re-

TABLE 3.—*Number of necrotic lesions obtained on 9 leaves of Nicotiana langsdorffii leaves rubbed with diseased tobacco juice which had been treated with commercial pepsin for various lengths of time*

| | | | | | |
|---|---|---|---|---|---|
| No. of hours treated | ¼ | 24 | 48 | 72 | 96 |
| No. of lesions obtained | 2500 | 430 | 290 | 140 | 38[a] |

[a] Virus from 10 single necrotic lesions was transferred to 10 healthy young tobacco plants.

duction in virus concentration, as measured by the numbers of necrotic lesions produced, due to digestion by the enzyme. Virus from 10 single necrotic lesions that resulted from infection with the 96-hour treated juice was transferred to young tobacco plants. Yellow spots appeared in each inoculated tobacco plant. By means of single pin-puncture inoculation, yellow-mosaic virus was shown to be present in at least 3 bright yellow spots.

In the preceding pages a number of experiments have been described in which attempts were made to obtain single infectious units of tobacco-mosaic virus. It is believed that numerous samples of virus derived from single infectious units were obtained and tested. In all instances tobacco plants infected with such samples produced, in addition to the ordinary symptoms of tobacco mosaic, bright yellow spots that contained yellow-mosaic viruses. The evidence obtained indicates that yellow-mosaic viruses arise in tobacco plants infected with tobacco-mosaic virus believed to have been derived from single infectious units.

### SUDDEN APPEARANCE OF NEW VIRUSES IN PLANTS INOCULATED WITH YELLOW-MOSAIC VIRUSES

It was observed that in sets of plants inoculated with any one of the various yellow-mosaic viruses, occasional plants suddenly exhibited symptoms different from other plants in the same set. Most frequently these sudden changes in symptoms were characterized by the appearance of green mottling of the tip leaves. Transfers of virus from such leaves produced symptoms in tobacco that resembled those produced by ordinary tobacco-mosaic virus infection. In a number of other instances a systemic yellow-mosaic infection suddenly appeared in plants inoculated with a slow-moving virus, which rarely produced symptoms on any leaf other than that inoculated. Owing to the fact that no control plants ever showed such symptoms of infection, it is believed that in each case the unusual symptoms were the result of infection with a new virus that suddenly arose from the virus originally transferred to the plant.

A number of experiments were designed to test further the possibility that these variations in symptoms might be due to contaminations. The general plan of the experiments was to inoculate 20 plants with a yellow-mosaic virus that was believed to be pure and free from other yellow-mosaic viruses. The 20 inoculated plants were surrounded by 20 control plants, the leaves of which were rubbed with healthy tobacco juice. When a slow-moving virus was transferred to the test plants, ordinarily no symptoms appeared on any leaves other than those inoculated, even though plants were held as long as 2 months. It is most improbable that any virus of the green-mottling type was continually present in these cultures. In several experiments, however, after plants inoculated with a slow-moving virus had been

held on the bench for 30 or 40 days, one or two plants suddenly exhibited mottling of the tip leaves. Green-mottling viruses were usually obtained upon sub-transfers from these leaves. Occasionally, yellow-mottling viruses were recovered from plants inoculated with slow-moving viruses. In all experiments the control plants showed no symptoms, nor was virus recovered from any of them, although repeated trials were made.

Other experiments carried out with yellow-mosaic viruses that produce systemic infection gave similar results, but, because of the type of infection, the results were not so striking. Green-mottling viruses were occasionally obtained from the tip leaves of plants infected with virus that had been repeatedly transferred by single pin-puncture inoculations, although all other plants of the sets were infected with only the yellow-mosaic virus and showed no divergent symptoms.

In addition to the isolated instances in which unusual symptoms developed in inoculated plants, another type of variation was observed. Now and then irregular-shape bright yellow spots appeared on leaves of plants mottled with yellow-mosaic virus. From two such spots viruses have been isolated that were different from those originally introduced into the plants.

It is concluded from these experiments that yellow-mosaic viruses originally isolated from yellow spots on plants infected with tobacco-mosaic virus sometimes give rise to other viruses.

## DIFFERENCES IN YELLOW-MOSAIC VIRUSES AND THEIR RELATION TO TOBACCO-MOSAIC VIRUS

During the course of these investigations, 51 samples of yellow-mosaic viruses have been isolated by means of single pin-puncture transfers from bright yellow spots on plants infected with tobacco-mosaic virus. The various yellow-mosaic viruses show great differences in (A) the intensity of the yellow symptoms, the amount of distortion, and the amount of necrosis produced in leaves of tobacco, (B) the intensity of yellow symptoms and the amount of stunting produced in tomato, (C) the type of infection, *i.e.*, necrotic or chlorotic, produced in *Nicotiana sylvestris* Speg. and Comes, (D) the relative speed of movement from point of inoculation, and (E) relative infectivity. On the basis of the above-mentioned symptom variations, it can be stated definitely that many, if not all, of the viruses isolated are different from tobacco-mosaic virus and from each other. Some of them have been transferred continuously over a period of more than 2 years and during this time have retained their original characteristics. With the exception of certain variations mentioned in the previous section, all viruses have retained their individual characteristics when repeatedly transferred or when held in dried leaves or frozen juice.

Although many, if not all, of the yellow-mosaic viruses isolated are different from each other, there are several lines of evidence demonstrating that

all of them are strains of tobacco-mosaic virus. The 51 yellow-mosaic viruses were isolated from leaves of plants infected with tobacco-mosaic virus derived from virus originally contained in two single necrotic lesions on leaves of *Nicotiana langsdorffii*. Chester (1), using both the precipitin and complement-fixation reactions, has demonstrated that about 20 of these viruses are serologically indistinguishable from tobacco-mosaic virus. All yellow-mosaic viruses produce infection in tobacco, tomato and *N. sylvestris*, and all produce necrotic local lesions on leaves of *N. glutinosa*. Many of the viruses have been kept in frozen juice or in dried leaf tissue for 2 years. Other viruses, isolated more recently, have not been tested in this connection. None of the yellow-mosaic viruses, with the possible exception of those of the "slow-moving" type, are inactivated by heating for 10 minutes at 80° C. All yellow-mosaic viruses are transmissible by mechanical methods. These properties and host reactions are characteristic of viruses belonging to the tobacco-mosaic virus group.

The evidence presented above indicates that many different yellow-mosaic viruses have been isolated from bright yellow spots on leaves of tobacco plants inoculated with virus of typical tobacco mosaic, and that all of these viruses are strains of tobacco-mosaic virus.

## DISCUSSION

Conclusive proof that variant strains of viruses arise from the tobacco-mosaic virus will be difficult to obtain until it is possible to separate with certainty single units of virus. Nevertheless, several lines of experimental evidence have been obtained, which, taken individually and collectively, strongly indicate that yellow-mosaic virus strains arise suddenly from the ordinary green tobacco-mosaic virus.

The most important evidence that variant strains arise from tobacco-mosaic virus is found in the fact that so many different strains showing some characteristics of tobacco-mosaic virus were isolated from plants infected with the virus of tobacco mosaic. Including experiments, presented in another report (4), 51 isolations of yellow-mosaic viruses have been made. Although only preliminary studies have been conducted on them, it is clear that many, if not all, differ from each other and from tobacco-mosaic virus. The strains have all been isolated from plants infected with virus originally present in one or the other of two single necrotic lesions. It seems altogether unlikely that virus of so many strains of yellow mosaic, in addition to the tobacco-mosaic virus, were present in each of these single necrotic lesions. Furthermore, the first tobacco leaves inoculated with virus from the single necrotic lesions developed no primary yellow lesions such as would have developed had the slightest trace of yellow-mosaic virus been intentionally added. The first indication that virus of yellow mosaic was present came

when a bright yellow spot appeared on a systemically diseased leaf of an inoculated tobacco plant. Some of the viruses isolated were not obtained until more than a year after virus in the single necrotic lesions was transferred, and, therefore, did not appear until many sub-transfers of the original virus had been made.

Yellow-mosaic viruses, difficult to transmit with ordinary rubbing methods of inoculation, were isolated from plants under conditions that practically precluded their having been present in the virus transmitted to the plants. In one experiment two different viruses, neither of which can be transmitted when diluted more than 1:100, were isolated from plants infected with tobacco-mosaic virus diluted 1:10,000,000.

In view of the evidence presented, it seems most improbable that the yellow-mosaic viruses are carried along in every single infectious unit of tobacco-mosaic virus. If viruses of such strains are carried along they must be linked in some manner with tobacco-mosaic virus and this bond must be broken when yellow-mosaic viruses appear in bright yellow spots.

It is of interest to note that, although viruses have been isolated that produce a wide range of symptoms, all are definitely related to tobacco-mosaic virus. In no instance have viruses been obtained having the properties of cucumber-mosaic virus, tobacco ring-spot virus, or any other virus unrelated to that of tobacco mosaic.

That the yellow-mosaic viruses, isolated from plants infected with virus of tobacco mosaic, in turn give rise to other yellow-mottling viruses or to green-mottling viruses similar to that of tobacco mosaic, is further demonstration that one virus may arise from a related virus. In such a demonstration the interrelationship of the different viruses of the tobacco-mosaic group is indicated and evidence of the variability of these viruses is strikingly brought out.

Regarding the origin of yellow tobacco-mosaic virus, McKinney (7) in 1929 stated that "it seems entirely possible that viruses may be altered locally in a plant, thus producing mutations, to use this term in its broadest meaning." Two years later in an abstract (8), McKinney stated that he considered the common mosaic of tobacco to be a "virus complex." Without presenting details of his experiments (9) he reported that he was unable to completely eliminate traces of yellow-mosaic virus in tobacco-mosaic virus, even by dilution and treatment with alcohol, acetone, acids, alkalis, high temperatures, ultra-violet radiation, centrifugalization, filtration, and inoculation to different species. McKinney (10) regarded the yellow and green mosaic of wheat as due to a mixture of two viruses, separable by repeated subinoculation. Jensen in 1933 (4) presented evidence from which he concluded that yellow tobacco-mosaic viruses arise during multiplication of green tobacco-mosaic virus in infected plants. Price (11), working with

cucumber-mosaic virus, brought evidence indicating that new strains of virus arise by mutation or some similar process in tobacco plants having the cucumber-mosaic disease. Hoggan (2) also obtained evidence of strain variation in cucumber-mosaic viruses.

Whether the new strains of virus that appear in infected plants should be designated as variant strains, mutant strains, viruses causing mutant symptoms, or by some other term is a question which cannot be settled definitely on the basis of our present knowledge.

SUMMARY

Experimental results were obtained yielding further evidence that yellow-mosaic viruses arise in plants infected with tobacco-mosaic virus. Attempts were made to obtain single infectious units of virus by means of high dilution, single pin-puncture inoculations, ultrafiltration, and chemical treatments. It is believed that many single infectious units of virus were obtained. All tobacco plants inoculated with virus derived from such units produced, in addition to the usual symptoms of tobacco-mosaic infection, bright yellow spots from which yellow-mosaic viruses were isolated.

In sets of tobacco plants infected with any one of the strains of yellow-mosaic viruses, occasional plants produced symptoms that were different from those produced by other plants of the same set. Sub-transfers of virus from tip leaves of plants showing unusual symptoms resulted in the isolation of virus unlike that originally introduced. It is believed that new viruses occasionally arise in plants inoculated with any of the yellow-mosaic virus strains.

Fifty-one isolations of yellow-mosaic viruses have been made. On the basis of variations in symptoms, rates of movement, infectivity, etc., it is concluded that many, if not all, of these viruses are different from each other. On the other hand, certain properties, host reactions, and serological relationships demonstrate that the yellow-mosaic viruses are strains of tobacco-mosaic virus.

The evidence strongly indicates that new strains of tobacco-mosaic virus arise suddenly in infected plants by some process apparently similar to that of mutation.

FROM THE DEPARTMENT OF ANIMAL AND PLANT PATHOLOGY OF
THE ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH,
PRINCETON, NEW JERSEY.

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# SEEDLING CULTURE IN SAND TO PREVENT DAMPING OFF

A. A. Dunlap

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Pure sand with inorganic salts added in solution has been found to be a suitable medium for the culture of many species of seedlings. This method eliminates most of the damping-off of seedlings so prevalent in soil culture. Furthermore, the growing of seedlings in sand makes possible certain desirable cultural practices.

It has been known that damping-off of seedlings is less apt to occur in light sandy soils than in those containing large amounts of organic matter. Sand also has been used to cover the soil surface in attempting to reduce the loss of seedlings through fungous attacks. However, by growing seedlings in pure sand, a medium is employed that, in the beginning, is entirely incapable of supporting the growth of most fungi. The only sources of organic material, necessary for fungous growth in such a culture, are later introduced with the seed material, or are formed by the growth of chlorophyll-bearing plants.

## MATERIALS AND METHODS

In this investigation the sand used was a brown sea sand from Long Island. It had a water-holding capacity of about 24 per cent. In regard to particle size, 30 per cent of the particles were less than 0.4 mm., 40 per cent were from 0.4 mm. to 0.8 mm., and the remaining 30 per cent were larger than 0.8 mm. in diameter. Good results also were obtained with sand of other types; however, a few kinds of sand from certain inland sources have been found unsatisfactory.

For the most part the cultures were grown in glazed crocks, 17 cm. in diameter, 10 cm. in depth, with a 1-cm. drainage hole in the bottom. Certain seedlings have been successfully grown in 2 or 3 centimeters of sand in water-tight containers, as well as in ordinary wooden flats. The cultures have been kept in the greenhouse except for certain trial cultures in cold frames and out-of-doors, which have shown results similar to those in the greenhouse.

The sand was washed in hot water (60° to 90° C.) until all sediment was removed, before filling the crocks. To each crock of sand was added 20 cc. of a 0.05 per cent solution of soluble $FePO_4$ by sprinkling. In the same manner 70 cc. of a nutrient solution with the following composition was added: $Ca(NO_3)_2$ — 2 g.; $KNO_3$ — 4 g.; $CaH_4(PO_4)_2$ — 1.2 g.; $MgSO_4$ — 1.2 g.; water — 1000 cc. Commercial grades of chemicals were used. A solution of higher than usual concentration was used to provide

the culture with sufficient nutrient to last through the seedling period with the usual watering and thereby make post-emergence fertilization unnecessary. Later applications of a more dilute solution were made when prolonged growth of the seedlings was desired. Nutrient solutions of this type gave pH readings of from 4.4 to 4.6, but, after addition to the sand, this reading was found eventually to be much higher. Experiments with other solutions in sand cultures have indicated that for most seedlings the type of nutrient solution is relatively unimportant as long as there are suitable amounts of nitrogen and potassium present. It appears possible for practical purposes that a 2-salt solution may be satisfactory in growing many kinds of plants through the seedling stage.

A definite number of seeds, depending upon the species, were placed in each crock after the addition of the nutrient solution. The seeds were covered with a layer of sand and the surface of the culture was then watered. Certain seeds that apparently require more oxygen in germination were covered with coarse sand or with sand mixed with such materials as asbestos or granulated charcoal. Enough sand was used in covering to hold the seedlings in place upon emergence, but not deep enough to seriously retard germination. Until the seedlings were ready for transplanting into soil, the only further attention necessary was the watering of the culture.

Soil cultures were similarly prepared and kept with the sand cultures under the same conditions and received the same amounts of water. The soil used in this work was for the most part a composted greenhouse soil containing light sandy loam, rotted manure, and leaf mold. The Rhizoctonia organism causing damping-off was present in the soil and a fungus of the Pythium type was found less frequently. Besides growing cultures of seedlings in the nontreated soil, other soil cultures were maintained with some of the more common soil and seed treatments. Some of the crocks of soil were autoclaved at 15 lbs. pressure for 2 hours, while others were treated with 20 g. of a 6 per cent formaldehyde dust. Certain seed treatments, such as cuprous oxide, Semesan, and zinc oxide also were used with nontreated soil and the results compared with the sand cultures.

## EXPERIMENTAL RESULTS

After the emergence of the first seedlings, each culture was examined daily for seedlings showing damping-off. The diseased seedlings were removed and the number recorded. When the seedlings had reached an ample size and were sufficiently mature for transplanting, they were removed from the substrate, counted, and weighed.

In table 1 are given the results of controlling damping-off by means of sand culture, expressed in terms of the number of healthy seedlings that had survived up to the time of transplanting, as compared with similar

TABLE 1.—*Comparison of seedling production in sand and in nontreated soil*

| Species | Number of seeds per culture[a] | Age of culture in days | Number of healthy seedlings | |
|---|---|---|---|---|
| | | | Soil | Sand |
| Aquilegia | 150 | 40 | 48 | 85 |
| Beet | 200 | 20 | 65 | 282 |
| Cabbage | 250 | 15 | 177 | 203 |
| Celery | 500 | 40 | 69 | 458 |
| Cosmos | 150 | 12 | 48 | 105 |
| Delphinium | 200 | 25 | 27 | 65 |
| Gypsophila | 500 | 20 | 87 | 404 |
| Lettuce | 300 | 15 | 51 | 267 |
| Pepper | 150 | 25 | 78 | 110 |
| Pine (white) | 100 | 50 | 9 | 34 |
| Salvia | 200 | 25 | 56 | 95 |
| Spinach | 250 | 20 | 43 | 127 |
| Spruce | 200 | 50 | 10 | 105 |
| Tomato | 150 | 20 | 96 | 118 |
| Zinnia | 150 | 15 | 41 | 85 |

[a] This number of seeds for each species was used in each culture throughout this work, either in sand or in soil.

culture in nontreated soil. This table also shows the number of seeds planted in each culture and the number of days from the time the seeds were planted until the final data upon the cultures were taken. These results include the averages of a large number of individual cultures grown at different seasons over a period of 2 years. It may be seen in this table that the survival of each of the 15 species of seedlings is consistently better in the sand than in the soil cultures grown under the same conditions. This increase in numbers of seedlings in the sand cultures varies from 12 per cent of the number of seedlings in the soil, in case of a less susceptible plant such as cabbage, to an increase of almost 1000 per cent in the case of spruce seedlings. The species listed in this table include both rapid-growing and slow-growing types, as is shown by the variation in the number of days (12 to 50) that the cultures were maintained. Cultures of seedlings in sand and in nontreated soil are illustrated in figure 1, A–E.

The average total numbers of seedlings that emerged in the sand and in nontreated soil cultures are shown in table 2. Here also is given the amount of post-emergence damping-off in both the sand and soil, expressed as the number of diseased seedlings per culture and also as the percentage of the total number of emerged seedlings. The number of plants emerged from the nontreated soil was generally much smaller than the number from similar sand cultures, because of preemergence damping-off in the soil.

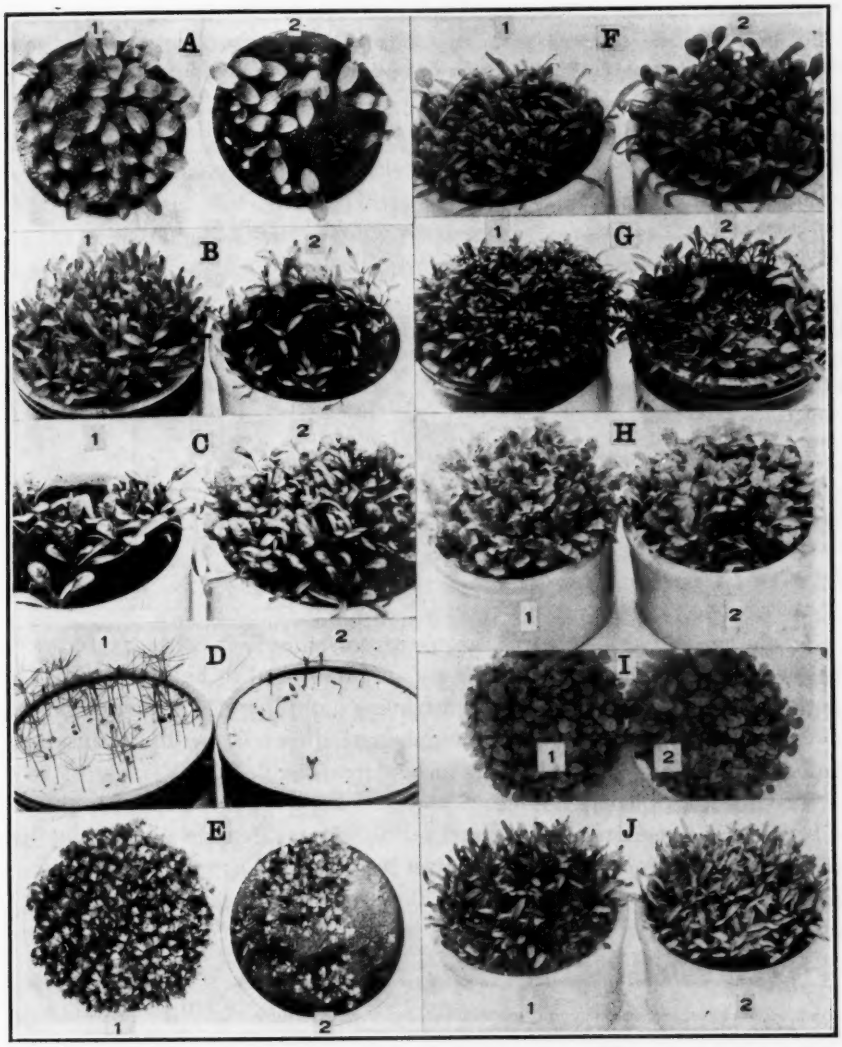


FIG. 1. Cultures of seedlings in sand and soil. A. Cucumbers: 1, sand; 2, soil. B. Beets: 1, sand; 2, soil. C. Spinach: 1, soil; 2, sand. D. Pine: 1, sand; 2, soil. E. Celery: 1, sand; 2, soil. F. Spinach: 1, soil with cuprous oxide-dusted seed; 2, sand. G. Gypsophila: 1, sand; 2, soil with formaldehyde dust. H. Lettuce: 1, sand; 2, soil with formaldehyde dust. I. Tobacco: 1, sand; 2, autoclaved soil. J. Beets: 1, sand; 2, autoclaved soil.

TABLE 2.—*Survival of seedlings after emergence, in sand and in nontreated soil per average culture*

| Species | Total number of seedlings emerged | | Seedlings damped-off after emergence | |
|---|---|---|---|---|
| | Soil | Sand | Soil | Sand |
| | | | *Per cent* | *Per cent* |
| Aquilegia | 65 | 85 | 26 | 0 |
| Beet | 177 | 291 | 63 | 3 |
| Cabbage | 186 | 205 | 5 | 1 |
| Celery | 193 | 458 | 64 | 0 |
| Cosmos | 52 | 105 | 8 | 0 |
| Delphinium | 50 | 67 | 46 | 3 |
| Gypsophila | 241 | 411 | 64 | 2 |
| Lettuce | 115 | 267 | 56 | 0 |
| Pepper | 97 | 110 | 16 | 0 |
| Pine | 24 | 35 | 63 | 3 |
| Salvia | 67 | 95 | 17 | 0 |
| Spinach | 102 | 132 | 58 | 4 |
| Spruce | 22 | 107 | 55 | 2 |
| Tomato | 104 | 118 | 8 | 0 |
| Zinnia | 53 | 85 | 23 | 0 |

In the case of soil cultures, with autoclaved soil or with treated seeds, the amount of preemergence damping-off was found to be greatly reduced. In these cultures, however, post-emergence damping-off frequently became so very prevalent that the final number of diseased seedlings was larger than the number of such seedlings in nontreated soil.

The results of comparisons of seedling survival in sand with those of soil cultures receiving heat sterilization, formaldehyde dust, and seed treatment with cuprous oxide are given in table 3. Cuprous oxide generally was found to be more effective than the other seed treatments used in this work. As compared with the results in table 1, the amount of damping-off

TABLE 3.—*Survival of seedlings in sand as compared with seed and soil treatments*

| Species | Number of healthy seedlings per culture | | | |
|---|---|---|---|---|
| | Cuprous oxide on seed | Formaldehyde dust in soil | Autoclaved soil | Sand |
| Beet | 174 | 155 | 260 | 282 |
| Cabbage | 184 | 195 | 217 | 203 |
| Celery | 388 | 397 | 421 | 458 |
| Gypsophila | 130 | 203 | 312 | 404 |
| Spinach | 104 | 79 | 135 | 127 |
| Spruce | 27 | 31 | 38 | 105 |

in the soil cultures was greatly reduced by the above treatments. With but two exceptions, however, the number of healthy seedlings is larger for the sand cultures than for the treated soil or seed. These two exceptions occur in the case of autoclaved soil and represent increases over the number of seedlings in the sand cultures of only 6 and 7 per cent respectively with cabbage and spinach. The results of the soil and seed treatments, together with comparable sand cultures, are illustrated in figure 1, F–J.

In these experiments, the most consistent damping-off control has been obtained by the sand-culture method. In each of the above treatments with soil, localized areas of damping-off infection have appeared in many individual cultures. In a limited number of cases, the cultures showing such infection were set aside and the damping-off in the soil cultures was found to spread until nearly all of the seedlings were destroyed, (Fig. 1, G2). In the sand cultures, loss through fungous attack has been found to be confined to individual seedlings or to a few seedlings growing closely together.

In general the individual seedlings grown in sand have compared favorably in regard to size, root development, and vitality with seedlings from soil cultures, although they may occasionally be somewhat lighter in color. A certain amount of variation has been noted among different species as to the relative rates of growth of the seedlings in sand and in soil. In certain cases the appearance of the sand cultures at the time of emergence of the seedlings has been inferior to that of the soil. After a short period of growth, however, the sand-culture plants may equal or even surpass those grown in soil in regard to size and fresh weight. Frequently the sand cultures have been found to produce the largest seedlings.

The percentage survival of seedlings transplanted from sand into soil has been found equal to that of comparable seedlings from soil. There have been no differences noted in subsequent growth and yield between plants produced by each of these methods. Transplanting from sand is more readily accomplished and the process may be made easier by flooding the sand with water. In this investigation certain sand cultures of seedlings have been maintained for extended periods before transplanting without the plants becoming too large or overcrowded.

An important advantage of the sand-culture method is the lack of care necessary in watering the seedlings. The sand may be given a large amount of water without danger of inducing damping-off. Consequently, less frequent watering may be required than would be necessary for soil culture. Another desirable feature is that the sand may be used indefinitely, with washing in hot water before replanting for the best seedling growth and damping-off control.

The following plants have been successfully grown through the seedling stage in these experiments, in addition to those listed in the preceding tables:

Alfalfa, bean, bent grass, brocolli, carrot, cauliflower, corn, cucumber, eggplant, endive, fennel, marigold, muskmelon, parsley, parsnip, pea, petunia, snapdragon, squash, tobacco, turnips, and watermelon.

## SUMMARY

Many different kinds of seedlings have been found to grow readily and develop normally in pure sand with nutrients added in solution.

Both preemergence and post-emergence damping-off have been much less in sand culture than in nontreated soil under the same conditions.

Seedling survival in sand culture has been found equal to that obtained in soil with the usual seed and soil treatments.

Certain cultural features, such as freedom from care in watering, ease of transplanting, and continued use of the substrate, are points in favor of sand culture for seedlings.

Seedlings grown in sand have been found to compare favorably with those grown in soil in regard to: rate of development, size, vigor, ability to withstand transplanting, and resistance to disease.

CONNECTICUT AGRICULTURAL EXPERIMENT STATION
NEW HAVEN, CONNECTICUT

# TOBACCO STREAK, A VIRUS DISEASE[1]

JAMES JOHNSON

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During the past 15 years the writer has repeatedly observed a disease of tobacco in the field that is characterized by a necrosis of, or along, the veins of the leaf. This disease was for many years believed to be one of the many nonparasitic leaf necroses to which tobacco apparently is subject. Usually the disease occurs on only widely scattered plants; only in one year is it recalled that it affected a sufficiently high percentage of plants to cause any concern among growers. Consequently, the disease has received no technical attention and does not appear even to have acquired a common name. The term "tobacco streak" is, therefore, proposed as perhaps the simplest and most suggestive for this malady. This disease has now been traced to a specific virus, herein described in what is believed to be sufficient detail to enable subsequent determination and differentiation from related viruses.

## THE DISEASE

While tobacco streak is not a common disease, isolated specimens usually may be found in most fields examined for its presence in Wisconsin. In its most severe form it may be found on as high as 100 per cent of plants in small portions of field borders, more particularly on areas bordering the farm-yard or weedy plots. No case has been observed where the disease occurred in large percentages throughout the field.

The disease under field conditions usually is confined to the leaves, but not all the leaves on affected plants show symptoms. The middle leaves are most commonly attacked and, as a result, may be considerably dwarfed and ragged (Fig. 1). The normal tendency is for the plant to recover from the attack to such an extent that the upper leaves on an affected plant may show no symptoms whatever.

The type of necrotic lesions produced is by no means uniform in character; spots, lines, and circles with or without uniform patterns occur, more often on the basal half of the leaf than on the apical half (Fig. 2). The leaves are rarely if ever entirely killed or dropped in field plants. In the greenhouse the leaves on young inoculated plants may be nearly destroyed under conditions most favorable for the disease, but, nevertheless, recover turgidity. Vein clearing, followed by "water soaked" necrotic systemic symptoms (Fig. 3) may appear on new leaves 3 days after inoculation,

[1] Cooperative experiments of the Wisconsin Agricultural Experiment Station and the Division of Tobacco and Plant Nutrition, Bureau of Plant Industry, United States Department of Agriculture.

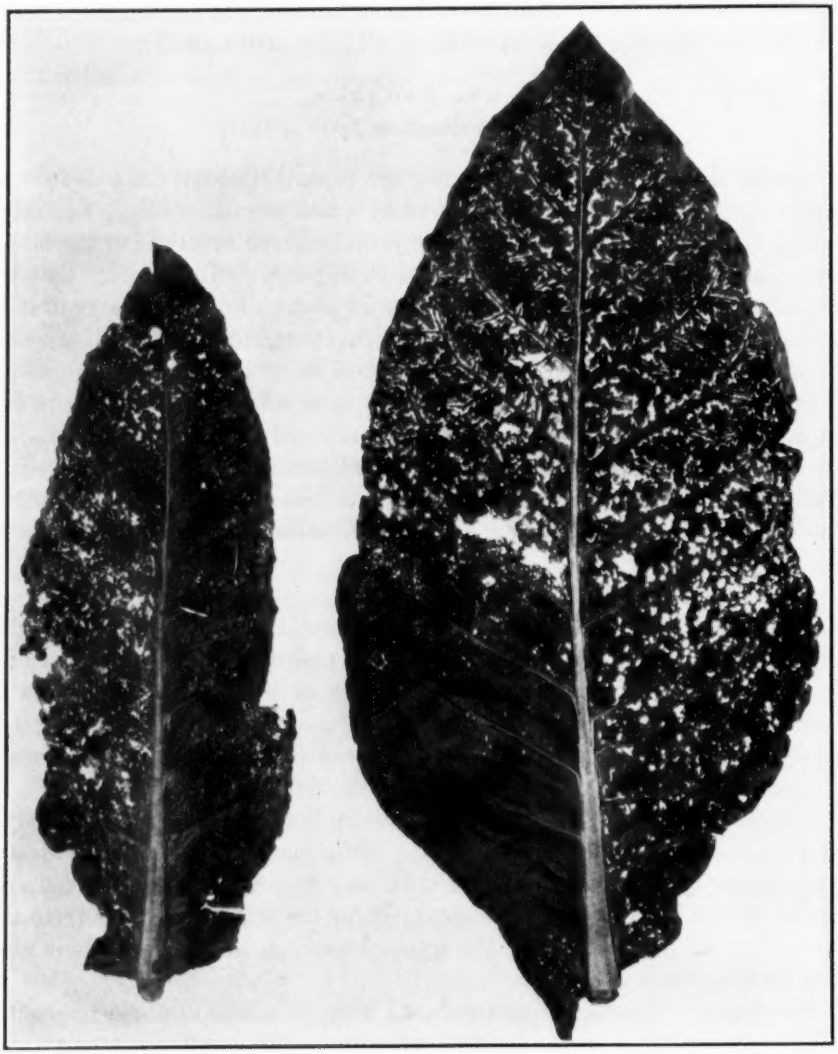

FIG. 1. Tobacco streak disease from natural field infection. The leaf to the left is greatly dwarfed as a result of early infection.

often preceded by local necrosis at the point of inoculation. Usually no mottling occurs either before or after the necrotic symptoms on tobacco. On young greenhouse plants, as in the field, the tendency for new leaves to recover is very striking, a condition that, though common with many virus diseases, is more marked in this than in any other disease observed. Recovery does not appear to bear any relation to the external environment of

FIG. 2. Tobacco streak. The relation of infection to both the coarser and the finer veins, and the progress of the symptoms toward the apex of the leaves is shown.

the plant. Reinoculation of recovered plants, in contrast to inoculated controls, results only in vein clearing and mild mottling of the recovered leaves, and the typical necrotic symptoms fail to appear. This is apparently a case of acquired resistance to the disease, or at least to the more severe form of disease. Investigations are being continued on this phase of the problem.

## THE VIRUS

*Transmission.*—The virus was collected from five different fields in September, 1934, and transmitted to young tobacco plants in the greenhouse without much difficulty. Previous attempts at transmission had generally

FIG. 3. Fairly typical symptoms of tobacco streak as secured on the leaves of young plants following artificial inoculation.

failed, probably because inoculum from old leaves was used. Extract from young leaves of field plants or from young suckers showing early stages of the disease gave a fair percentage of infection, and subsequent transfers from young greenhouse plants invariably yielded 100 per cent infection on Havana-seed tobacco by means of the rubbing method of inoculation. The virus evidently is not so infectious as the ordinary tobacco mosaic virus, since it does not appear to be spread extensively in the field through cultural operations.

The localization of the disease to field borders suggests transmission of the virus into the field from some near-by host through the medium of some insect vector. A similar observation as to field-border relations has been made by Thung (9) for the "curl" disease of tobacco in Java, which virus is transmitted by a species of white fly.

Although opportunity has not yet been afforded to examine such infested areas for insects, a limited number of tests have been made with certain probable vectors that were obtainable for the trials. From these tests it may be stated with considerable certainty that neither our more common aphids nor the greenhouse white fly are involved, and more limited attempts with thrips and leafhoppers have been equally unsuccessful in transmitting the virus. The following insects were used in these trials: *Myzus persicae* Sulz., *Myzus pseudosolani* Theob., *Macrosiphum solanifolii* Ashm., *Aleyrodes vaporariorum* Westw., *Thrips tabaci* Lind., a greenhouse thrips (undetermined species), and *Empoasca fabae* Harris.

Field observations do not suggest any possibility of transmission through seed.

*Host Range.*—No attempts have been made to transmit tobacco streak virus to plants outside the family Solanaceae. The following Solanaceae have been infected in greenhouse trials: *Nicotiana tabacum* L., *N. rustica* L., *N. glutinosa* L., *N. tabacum* × *N. glutinosa* hybrid, *Datura stramonium* L., *Nicandra physalodes* (L.) Pers., and *Physalis pubescens* L. Tomato (*Lycopersicon esculentum* Mill.) evidently is immune from infection, as is potato, *Solanum tuberosum* L.; egg plant, *S. melongena* L.; and pepper, *Capsicum annuum* L. Distinct mottling and sometimes necrosis occur on *N. glutinosa,* and mild mottling may occur on *Datura stramonium.* Since these latter hosts are attacked only locally by the ordinary tobacco-mosaic virus, whereas the streak virus may become wholly or partially systemic, they made possible a reisolation of the streak virus after this had become accidentally mixed with the tobacco mosaic virus.

*Properties.*—The tobacco-streak virus is relatively short-lived in extract. In 3 trials it usually was inactivated after aging for 24 hours in extract at approximately 22° C. In one trial it survived 24 but not 36 hours. Its longevity *in vitro,* therefore, may be placed at between 24 and 36 hours (Table 1). The thermal deathpoint was found to be 53° C. for 10 minutes (Table 1), and the tolerance to dilution, while not accurately determined, was low, the virus barely surviving a dilution of 1 in 20 and, probably, not extending beyond 1 in 30 (Table 1). Taking all properties into consideration, it is clear that the streak virus is very sensitive to external conditions, and, in the absence of vegetative propagation of its host, a very limited survival of the virus may be expected from year to year. On the basis of the studies conducted, the following technical description is offered for the virus causing the tobacco streak disease.

*Tobacco Streak Virus.*—Not transmitted by *Myzus persicae, Myzus pseudosolani* nor *Macrosiphum solanifolii.* Transmissible mechanically by plant extract. Resistance to aging *in vitro* between 24 and 36 hours at 22° C. Thermal deathpoint 53° C. at a 10-minute exposure. Tobacco, *Nicotiana tabacum* L., susceptible, developing local and systemic necrotic lesions on leaves, the latter symptoms being closely associated with the veins. Recovery of upper leaves is a striking characteristic of the disease of tobacco. Mottling and sometimes necrosis on *N. glutinosa* L. No infection on tomato, *Lycopersicon esculentum* Mill.

## DISCUSSION

On the basis of brief symptom descriptions and illustrations to be found in the earlier literature on tobacco diseases, it is not unlikely that the disease here described as "tobacco streak" has been previously observed elsewhere as a disease of unknown cause. More recently, several virus diseases of tobacco of the necrotic type have been described, but the descriptions in

TABLE 1.—*Determinations on certain properties of the tobacco streak virus*

| Longevity *in vitro*, 22° C. | | | | Thermal death point (10 min.) | | | | Tolerance to dilution | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Hours aged | Number of trials | Number of plants inoc. | Number of plants inf. | Temp. ° C. | Number of trials | Number of plants inoc. | Number of plants inf. | Dilution | Number of trials | Number of plants inoc. | Number of plants inf. |
| 0 | 4 | 20 | 20 | 0 | 7 | 35 | 34 | 0 | 6 | 30 | 30 |
| 6 | 3 | 15 | 13 | 50 | 4 | 20 | 17 | 1–5 | 2 | 10 | 7 |
| 12 | 4 | 20 | 15 | 52 | 2 | 10 | 5 | 1–10 | 6 | 30 | 10 |
| 24 | 4 | 20 | 5 | 53 | 3 | 15 | 0 | 1–20 | 2 | 10 | 2 |
| 36 | 3 | 15 | 0 | 54 | 2 | 10 | 0 | 1–30 | 2 | 10 | 0 |
| 48 | 3 | 15 | 0 | 55 | 4 | 20 | 0 | 1–100 | 4 | 20 | 0 |

some instances have remained too incomplete as to symptoms or too limited as to the virus itself to permit a reliable comparison. While it can be definitely stated that "tobacco streak" is not to be confused with such necrotic virus diseases as tobacco ring spot (2), spotted wilt of tobacco in Australia (7), or the "Kromnek" disease in South Africa (6), it is not so definitely distinguishable at present from the Rotterdam B disease of Sumatra (3), the "vein-streak" of Sumatra described by Jochems (4), the "curl" disease of Storey (8), the "stripe and curl" of Böning (1), the "kroepoek" disease of Thung (9), or the "tobacco etch" diseases of Kentucky (5). Of the above named, the disease most closely resembles "vein-streak" of Sumatra, and, with this similarity in mind, the term "tobacco streak" has been retained for the American disease here described. As the methods and technique for virus descriptions improve, it is expected that more satisfactory comparisons of these diseases may be more readily made.

Fortunately, tobacco streak is not a sufficiently serious disease in tobacco fields to warrant any great concern about the best means of control. The discovery of the nature of the disease, however, should aid greatly in making reliable recommendations in this direction. It appears fairly clear that this virus cannot overwinter in the soil, and the localization of the disease in the fields seems to preclude any likelihood of transmission through the seed. Everything considered, it seems most likely that the virus is harbored by perennial weed hosts in the neighborhood of the tobacco fields. Considering the frequency of the occurrence of the disease, even though in very limited amounts, the alternate host must be a fairly common one, and it should not prove difficult to locate such an overwintering host if it actually exists. The transmitting agent from such a possible host or hosts also remains unknown. It is clear that experiments on the control of the disease might well be directed toward the discovery of a possible overwintering host and a natural transmitting agent.

## SUMMARY

Tobacco "streak," a relatively rare disease of tobacco, is shown to be caused by a virus. The disease is characterized by striking necrotic leaf patterns, usually bearing a close relation to the veins. The disease is not new, but the nature of the malady has remained obscure in the past.

The virus is mechanically transmissible by plant extract, but no insect vector has been found. The properties of the virus show that it is relatively sensitive. It withstands aging *in vitro* less than 36 hours, the thermal death point is 53° C., and the tolerance to dilution is less than 1 in 30. Several other solanaceous species are susceptible to the virus, while others, like the tomato, apparently are immune.

The control of the disease probably will remain dependent upon the discovery of a natural overwintering host and a possible insect vector.

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## PHYTOPATHOLOGICAL NOTES

*A Method for Staining Rust Mycelium in Woody Tissues.*—During the course of a study of Scotch pine, infected with a species of Peridermium, the author[2] had occasion to use with good success the orseillin BB-anilin-blue-staining procedure described by Strasburger, pp. 392 and 767.[4]

The staining of rust mycelium and haustoria in tissues was so effective that the method suggested itself as valuable in the routine microscopic diagnosis of the white-pine blister rust. The method perhaps most often used for this purpose at present is the short method described by Colley[1] with the use of safranin and light green. The Strasburger procedure has been adapted to the shorter Colley method, so that sections may be stained in a comparatively short time.

Sections of white pine bark and wood, cut on a freezing microtome or by hand, are placed in water without previous fixation. The water is replaced by a saturated solution of orseillin BB[3] in 3 per cent acetic acid that is allowed to remain for 10 to 12 hours. The sections are then rinsed in 40 per cent ethyl alcohol in order to remove excess stain. A saturated solution of anilin blue in 3 per cent acetic acid is then added and allowed to remain for 15 to 30 minutes. A rapid rinsing in 40 per cent ethyl alcohol to remove any excess stain is followed by the addition of 95 per cent alcohol for 2 to 5 minutes. This is followed by absolute alcohol, which in turn, is replaced by fresh absolute alcohol. The sections may then be transferred to clove oil in which they may be examined if the mount is to be temporary, only. The clove oil may be washed away by xylol, and balsam or gum damar added if the sections are to be mounted permanently.

The most satisfactory results are usually obtained when differentiation with 40 and 95 per cent of ethyl alcohol has been carried on, so that the sections appear a rather light purple. It will be found necessary to alter the time schedule somewhat according to the thickness of the sections and according to the amount of resin the pine contains, as portions abundant in resin stain heavily with orseillin.

In properly differentiated tissues the mycelium stains violet to blue, lignified or suberized tissue stains red, parenchyma cell walls usually blue,

[1] Colley, R. H. Diagnosing white-pine blister-rust from its mycelium. Jour. Agr. Research [U. S.] 11: 281–286. 1917.

[2] Hutchinson, W. G. Resistance of *Pinus sylvestris* L. to a gall-forming Peridermium. Phytopath. 25: 819–843. 1935.

[3] Orseillin BB may be obtained from Akatos Inc., 55 Van Dam St., New York, N. Y. It is not the same stain as orsein.

[4] Strasburger, E. Das botanische Praktikum. 7th Ed. 883 pp. G. Fischer, Jena. 1923.

and nuclei and cytoplasm red. Haustoria, penetrating tracheids and surrounded by lignified callosities, stain deep red in contrast to the bluish mycelium outside the cell wall.

Comparison of blister-rust-infected tissues stained by the above method with similar tissues stained with safranin and light green shows that in the former case the mycelium is much more obvious and clear-cut. Even in dried woody tissues, it is a simple matter to recognize the rust mycelium.—W. G. HUTCHINSON, Department of Botany, University of Pennsylvania, Philadelphia, Pa.

*The Status of the Nematode Aphelenchus avenae Bastian, 1865, as a Plant Parasite.*—There are considerable numbers of nematode species other than obligate plant-parasitic forms, which are found associated with diseased plants more or less frequently,—some even regularly. Their status as disease agents is in most instances doubtful. Most of these forms are thought to be connected only with decay, a viewpoint which is supported by the fact that they can exist in or on decayed material and do not need healthy tissue, as do the obligate plant parasites. However, observations and a few experiments seem to prove that this view does not entirely cover the actual situation. The connection of these forms with various diseased conditions in plants is of a more serious nature than is generally conceived. It may be that many of these forms live as saprophytes, but they undoubtedly are able to attack and to damage healthy plant tissues and to propagate in tissue not yet decayed. Contrary to the views generally expressed in this regard[1] concerning the significance of *Aphelenchus avenae,* our observations in numerous instances have proved again and again that *A. avenae* is not a harmless form. In diseased plant material submitted to the Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture, *Aphelenchus avenae* is one of the most frequently observed species, often associated with other nemas but often also found as the only form present. It may perhaps live on necrotic plant tissue, but it undoubtedly can live on fungi and on healthy plant tissue. This form is to be considered as a facultative plant parasite with perhaps dominant necrobiotic food tendencies. To substantiate this view a sagittal section through the root of a phlox hybrid[2] is illustrated, showing in the parenchyma of this root tissue 2 specimens, a larva and an adult female of *A. avenae,* with 5 eggs scattered through the tissue, one of the eggs apparently having just been deposited (Fig. 1). *Aphelenchus avenae* undoubtedly migrates through the tissue and, of course,

[1] Goodey, T. The pathology and aetiology of plant lesions caused by parasitic nematodes. 34 p. Imp. Bur. Agr. Parasitol. St. Albans, Eng. 1935.

[2] Received through the courtesy of Mrs. Flavia H. Moise, of the U. S. Dept. of Agriculture. The plant is a hybrid of *Phlox glaberrima suffruticosa.*

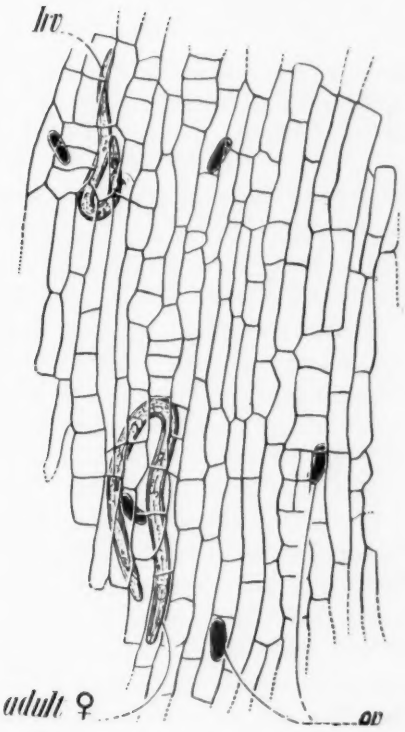


FIG. 1. *Aphelenchus avenae* female, larva and eggs in situ in parenchyma of cortex of phlox root; lrv larva; ov eggs. × 80.

in this way must inflict mechanical injury. It is thought that it feeds on the cell contents of the host tissue, but it is unknown whether only a certain part of the cell content is sucked in or the whole. In addition, the waste products of its metabolism are discharged into this plant tissue and no doubt interfere with its normal functions. The fact that the female specimen figured had deposited at least 5 eggs throughout the tissue undoubtedly proves that the nematode is able to sustain life there and is in this instance a parasite.—G. STEINER, Bureau of Plant Industry, U. S. Dept. of Agriculture, Washington, D. C.

## BOOK REVIEWS

Curtis, Otis Freeman.—*The Translocation of Solutes in Plants: A Critical Consideration of Evidence Bearing upon Solute Movement.* xiii + 273 pp. Illus. diagrs. McGraw-Hill Book Co., Inc.: New York and London. 1935. $3.00.

Professor Curtis, after 17 years of work on problems associated with translocation of solutes in plants, has at last assembled his material in book form. It will be recalled that, while orthodox phytophysiological dogma includes among its tenets the doctrine that mineral salts and other soluble materials (including sugars) travel upward from the roots to the growing organs through the xylem and that the manufactured foods (carbohydrates and proteins) travel downward from the leaves to the roots via the phloem, there are some heterodox iconoclasts among plant physiologists who do not subscribe to this particular article of faith; included in this small group of unbelievers are H. H. Dixon and O. F. Curtis.

The former holds that the xylem is the chief channel for both upward and downward movement, and relegates the phloem to a rather unessential role as far as translocation is concerned. Curtis, on the other hand, while admitting that the xylem does have a function in carrying water from the roots to the leaves, believes that the phloem carries nitrogen compounds and salts upward as well as other soluble nutrients and foods, in addition to its commonly accepted function of downward transportation. The seven chapters of this book set forth the author's reasons for this belief, with an explanation of how this double-dealing is accomplished and a short but pertinent discussion of some cognate problems.

After a brief introduction (Chap. I), the author launches into a presentation of the evidence for the upward transport, through the phloem, of organic matter (Chap. II) and of nitrogen and salts (Chap. III). These two are by far the most important and interesting chapters in the book. They contain the essence of the Curtisian creed, and include a comprehensive description of the author's ringing experiments, which certainly indicate that, in the plants described and under the conditions given, the phloem carries the burden of the substances under discussion. There seems to be no other alternative. The problem is too complex to consider in detail here, although one cannot help but wish that more space were given to an explanation (or refutation) of the classic experiments upon which the orthodox beliefs are based. It is easier for one to believe Curtis' explanation of his particular experiments than it is to throw overboard the more conventional ideas; in other words, it still remains to be shown that his conclusions are of universal application and can be generalized for all plants and under normal conditions.

There follows a confutation (Chap. IV) of the Dixonian doctrine, mentioned above; and here Curtis will have with him most of his readers. At this point is then presented (Chap. V) the criteria for determining tissues of transport, wherein it is pointed out that the more commonly accepted criteria for determining the region of translocation, such as the movement of solutes introduced through cut tissues or the presence of a substance in a tissue, are not valid in that they do not *prove* that these tissues conduct the materials in question. This is all too true, but physiological science still contains so many unknowns that "sweet reason" yet remains a virtue; and it is not hard to see that Curtis is a little more severe in demanding *absolute* proofs when treating the theories he is seeking to displace than when dealing with his own hypotheses.

But by this time the reader is supposed to be convinced that the phloem is the logical (and experimentally proved) channel of both upward and downward translocation. The question as to *how* this is accomplished is answered in the following sections (Chap. VI). In this excellent discussion of the problems involved, including a critique of the contributions and theories of Münch and Crafts, it is suggested that the chief factor in translocation is some sort of streaming and rotational movement of the protoplasm in the phloem (sieve tubes), which occurs in such a way that descending materials are fed in at the tops of the cells and ascending materials in at the bottom. While such a system seems possible, the difficulties are so great that one can only conclude that if plants don't use the transpiration stream in the xylem for their ascending materials they are missing a big chance to save themselves a lot of trouble!

While readers, in general, will approve of the author's discussion and conclusions, it seems to the present reviewer that Curtis has not always distinguished carefully (*e.g.*, in discussing Münch's hypothesis) between mass diffusion and osmotic gradients. The former deal primarily with proteins and other materials of such molecular size (colloids) that they do not exert osmotic pressure. *Osmotic* gradients, therefore, do not enter into the question of the diffusion of proteins, and the objection that such materials in passing downwards from the leaf go "against the osmotic gradient" does not hold. Also one must not forget that each substance exerts its own osmotic pressure independently of the others present; it is hence quite possible for the total osmotic pressure in "receiving cells" to be higher than that in "supplying cells" (see p. 152) and yet for the concentration of some individual component, *e.g.*, $KNO_3$, to be lower in the former. Furthermore, one must question the statement on page 151 that "Although in trees there seems to be a tendency for an increase in osmotic concentration of leaves at greater heights, this increase is not uniform, is at best not very great, and seems to bear no relation to the distance through which foods must move."

Dixon and Atkins (1916) found at the end of February an increase of osmotic pressure in the wood of *Acer macrophyllum* of about 0.2 atmosphere for each meter of elevation above the ground; and Harris and his coworkers, as well as others, have found considerable (and regular) increases in the osmotic pressure of leaves as one goes from the base of the crown to the top.

In a final chapter (VII) is discussed the relation of the translocation of solutes to such kindred questions as the movements of hormones, polarity, and regeneration, these phenomena being interpreted in the light of the hypothesis of Curtis. An extensive (indexed) bibliography and subject index conclude the volume.

From the point of view of the plant pathologist, the principal subject discussed is the movement and path of viruses. Evidence reviewed seems to show that the viruses commonly are carried in the phloem. Results of some workers indicate an association of the virus movement with that of carbohydrates and that the movement is unidirectional; but still other results show the movement to be independent of the movement of nutrients and to be in both directions at the same time. There would, hence, seem to be no uniformity in this matter; each virus has a specific behavior.

The book is splendidly written and printed, with few typographical errors, *e.g.*, an incorrect spelling of *pseudoacacia* on p. 161 (spelled correctly, however, on p. 180). The style is clear-cut; on the whole, logical; concise; and eminently readable. The summaries of the various sections at the end of each chapter are decided aids to the reader in extracting the essence of the text, and are models of condensation and of the abstractor's art. Few writers seem able to summarize their own work so admirably. But why do the publishers print "First Edition" on the title-page? Isn't it understood that, unless otherwise stated, the edition is the first? Or is this intended as an implication that there are more to follow?—ORAN RABER, Plant Physiologist, Forest Service, U. S. D. A.

Wardlaw, Claude Wilson. *Diseases of the Banana and of the Manila Hemp Plant*. 615 pp. 2 col. pl. illus. Macmillan & Co., London, 1935. $8.00. (30 s.).

The author of this work indicates in his preface that precise knowledge of the maladies affecting bananas is far from complete and that the task of selecting it from the voluminous but scattered mycological and agricultural literature on bananas has been one of no little magnitude. He has accomplished the task with rare discrimination and has presented an admirable summary of reliable contributions on this important crop. The book is carefully edited and abundantly illustrated with photographs and line drawings, including many from his own original papers. About a fourth of the large volume is devoted to banana wilt or Panama disease caused by *Fusarium*

*oxysporum cubense*, the most widely known and injurious disease of bananas in the American tropics. In addition, plantation diseases of the fruit and leaf, and storage diseases of the fruit are exhaustively treated. Somewhat briefer reviews of the diseases of abacá and deterioration of the fiber are presented. The most recent as well as the earlier literature has been assembled and sifted in the light of up-to-date research; and the information presented represents current thought of investigators in the field of banana husbandry and transportation. An interesting feature of the section on banana wilt is a short account of attempts to breed for resistance, which is complicated, because the banana is a sterile plant propagated by vegetative offsets. Reproductive organs of both sexes are abortive to an extent of more than ninety-nine per cent. Success of the program of improvement by breeding depends upon the fact that seeds may develop under special conditions, but it is essential of course that the new varieties intended for commercial use do not have seeds. There is reason for believing that, when grown in pure stands, even the small percentage of normal sexual cells formed have no chance of functioning, because of self-sterility. Resort was had in the earlier work to crossing with the highly fertile pollen of seed-bearing species of Musa. Hybrid seedlings, apparently immune from wilt, were produced in this way, but the disadvantage is the presence of an occasional seed. Cytological observations make it reasonable to conclude that triploid seedlings of commercial usefulness may be produced by synthesis from diploids or by reduction from tetraploids.

The author concludes the book with four appendices containing lists of bacteria and fungi associated with the banana plant as saprophytes and parasites, cultural characteristics of strains of *Fusarium cubense*, statistics on imports of bananas into Great Britain, and data on environmental conditions in the holds of banana boats. Without question, he deserves credit for one of the best presentations of disease hazards and control measures to be found in plant pathological literature relating to tropical crops.—E. W. BRANDES, Bureau of Plant Industry, Department of Agriculture, Washington, D. C.

Tehon, Leo Roy. *A Monographic Rearrangement of Lophodermium*. 151 pp. Illinois Biological Monographs, Vol. XIII, no. 4. (Univ. of Illinois, Bul. v, 32, no. 51, Aug. 20, 1935.) 1 illus. 5 plates. 1935. Price $2.00.

The bringing together in one accessible publication of a large body of data scattered through the mycological literature of many countries and covering a century or more is an undertaking to be commended, even though one is not able to agree with all phases of the treatment as presented by the author. The publication under review covers a detailed study of the mate-

rial available to the author of the genus Lophodermium here assigned to the family Hypodermataceae. The author points out that the genus is one that has not been thoroughly investigated as to its taxonomic relationships, and he gives a summary of studies made to date by other mycologists, with special reference to the work of Fries, Rehm, von Hoehnel, and Darker. A very detailed account of the morphology of the hysterothecium and other structures involved, based to a considerable extent on the author's own studies, follows.

In the systematic section constituting the larger portion of the paper 4 new species and one new combination are recognized in the genus Lophodermium proper, with one species renamed. As segregates from Lophodermium the author names as new the genus Dermascia and takes up two names proposed, but not described by von Hoehnel, namely, Lophodermellina and Lophodermina. These segregations are based in large part on the controversial von Hoehnel system, which uses the general position of the ascocarp with relation to the host tissue, *i.e.*, whether subepidermal or intraepidermal, as a basis of generic distinction. In Dermascia, one new species and 13 new combinations are made, in Lophodermellina one new species and 5 new combinations, in Lophodermina 2 new species and 20 new combinations. Descriptions, both of the several genera discussed and the species themselves, appear to be complete and carefully drawn and few typographical errors have been noted. A host list enhances the value of the publication to phytopathologists.—J. A. STEVENSON.

# REPORT OF THE OFFICIAL REPRESENTATIVE COMMITTEE OF THE SOCIETY, SIXTH INTERNATIONAL BOTANICAL CONGRESS AND INTERNATIONAL UNION OF BIOLOGICAL SCIENCES, SEPTEMBER 1935, AMSTERDAM

*International Union of Biological Sciences.* Adherence of the United States to this International Union was made possible by action of the National Research Council during the summer of 1935. It fell to members of your committee to cast the votes of the United States at the Amsterdam meeting on September 1, due to the fact that no other American societies had arranged for accredited representatives.

An American botanist, Doctor E. D. Merrill, of Harvard University, (recently of the New York Botanical Garden) was elected president of the Union.

A subsection of phytopathology was authorized in the Botanical Section. Doctor Donald Reddick, Cornell University, was elected Vice-President of this subsection.

Several other subsections were authorized including a Subsection of Applied Botany. The latter was authorized with the distinct understanding that it should not include phytopathology. Your representatives voted against the organization of this subsection and also against the organization of a Subsection on Forestry which was defeated.

*Sixth International Botanical Congress.* Members of your committee attended all sessions of the Subsection on Nomenclature, including the special sessions held by the mycologists, as well as all sessions of the section on phytopathology. The results of all official actions taken at the various meetings will be published elsewhere in detail.

The actions of chief interest to this Society were:

(1) *General Resolution.*

It is proposed that the Botanical Section of the International Union of Biological Sciences should act as an administrative connecting link between the successive International Botanical Congresses, which maintain their full independence from an international point of view to such an extent that any country which has not joined the Union, will have quite the same rights as those which have joined.

It is proposed that this Botanical Section be authorized to carry through any resolutions, carried by the International Botanical Congresses.

Proposed by A. C. SEWARD and seconded by J. C. SCHONTE, E. J. BUTLER, E. D. MERRILL, N. NĚMEC, M. J. SIRKS and N. E. SVEDELIUS.

(12) *Sections Myc. and Path.*

The Sixth International Botanical Congress wishes to express its sincere appreciation of the admirable work accomplished by the Centraalbureau voor Schimmelcultures at Baarn since its foundation in 1906 and views with grave concern the present financial difficulties of what is essentially an international institution.

Proposed to the Section MYC by J. RAMSBOTTOM and seconded by E. J. BUTLER; to the Section Path. proposed by E. RIEHM and seconded by E. C. STAKMAN.

(13) *Section Path.*

The Committee on Description and Nomenclature of Plant Viruses appointed by the Fifth International Botanical Congress 1930 wishes to report that it has made progress in developing a scheme for the nomenclature of plant viruses and suggests to this congress that the Committee be empowered to continue its consideration and establish an acceptable system of virus nomenclature.

Proposed by H. M. QUANJER and seconded by JAMES JOHNSON, P. A. MURPHY, J. HENDERSON SMITH and G. SAMUEL.

(14) *Section Path.*

It is recommended that the term "physiologic race" be substituted for "physiologic form" as the former seems more appropriate. It is recommended further that the word "race" be used in general to designate biotypes or groups of biotypes that differ from each other in physiologic characters.

Proposed by E. C. STAKMAN and seconded by Miss M. NEWTON, Miss G. MILBRINK and TH. ROEMER.

(15) *Section Path.*

The Sixth International Botanical Congress expresses itself of the opinion:

That an effective and unceasing campaign against destructive plant diseases, insects and pests can be successfully prosecuted only by international action and mutual cooperation;

that close and frequent international discussion of the problems of plant quarantines should take place to bring about improvement of the health conditions of plants and plant products offered for export;

that it unanimously recognizes that such action will greatly facilitate international trade in the commodities concerned, and

that, finally, this resolution be brought to the attention of the League of Nations, emphatically endorsing the League's proposal to give this matter the urgent and careful consideration with a view to facilitating and expediting the purpose and aims of this resolution.

Proposed by H. T. Güssow and seconded by N. VAN POETEREN.

*The Seventh International Botanical Congres* will be held in Sweden in 1940.

For the Committee,

H. T. Güssow, *Chairman.*